



सत्यमेव जयते

INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI

I.A.R.I.6.

GIP NLK—H 3 I.A.R.I.—10-5 55—15,000

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

JANUARY, 1949

NUMBER 1

CANADIAN CHEDDAR CHEESE FROM PASTEURIZED MILK

J. J. JANZEN AND R. W. BROWN

Department of Dairy Science, The University of Manitoba, Winnipeg

INTRODUCTION

In Canada, pasteurization in the dairy industry has been adopted very generally except in the manufacture of Cheddar cheese. However, because a number of epidemics of typhoid fever occurring in recent years in different parts of Canada have been traced to infected Cheddar cheese, the making of this kind of cheese from pasteurized milk has become an issue. Outside of the economies involved in the application of pasteurization to cheese making, the chief difficulty lies in the belief, among some authorities, that pasteurization sacrifices the typical Cheddar cheese flavor.

There were two chief objects in making this study. The first and foremost was to find out how well Cheddar type cheese made from pasteurized milk would meet Canadian market requirements, as indicated by the Canadian system of scoring and grading cheese by officials of the Marketing Service of the Dominion Department of Agriculture. Until now, no extended study of this nature has been made. The second object was the employment of pasteurization processes which would produce effective results according to the phosphatase tests and thus meet public health requirements in this regard.

While the public health measure in Canada, requiring the holding of cheese for 90 days before being sold to the consumer, applies both to cheese made from pasteurized and unpasteurized milk, the inclusion of pasteurized milk cheese under this regulation is not an issue at present because no factory in Canada is yet using pasteurized milk for Cheddar cheese. If and when this should be done, the authors believe it should be feasible to protect the milk and the curd from recontamination by employing common medical and sanitary measures in the factory.

HISTORICAL

A number of epidemics, traced to Cheddar cheese, have been reported in recent years (1, 3, 5, 9). Bowman (1) reported three separate outbreaks of typhoid fever in Manitoba, traced to Cheddar cheese. Gauthier and Foley (5) reported a large outbreak in Quebec and recommended pasteurization of cheese milk and a minimum holding period of 3 months for raw milk cheese prior to selling such cheese to the consumer. During 1945, 6 of the 24 reported epidemics traced to milk and milk products in the U. S. A. were due to Cheddar

Received for publication June 14, 1948.

cheese (3). Fabian (4) presents a well balanced review and discussion of disease outbreaks traceable to cheese.

Campbell and Gibbard (2) found that, under experimental conditions, most of the *Eberthella typhosa* organisms were dead in cheese held at 58–60° F., after a storage period of 3 months. However, similar cheese held at 40–42° F. retained viable typhoid organisms for at least 6 and in a majority of cases for 10 or more months.

Price (7, 8) reports that pasteurization of milk for cheese making results in an increased yield and improves the uniformity and keeping quality of the cheese. Review of earlier literature and experimental work is well covered in one of these papers (7). Tuckey (9) reports results which are in close agreement with those of Price (7) with respect to yield and keeping quality of the cheese. Irvine *et al.* (6) reported no increased yields where pasteurized milk was used. However, they did notice a significant improvement in butterfat retention in the pasteurized milk cheese.

Walter and Lochry (10) found that no. 1 cheese was increased where pasteurization was employed even though the no. 1 production was high prior to pasteurization. Wilson *et al.* (12) found that cheese made from pasteurized milk of good quality and with proper control of acid development could be ripened safely at a temperature high enough to permit the continued activity of essential bacteria.

EXPERIMENTAL PROCEDURE

All the experimental cheese was made during the months of June and July in the Department of Dairy Science, The University of Manitoba. Small vats holding 450 lb. of milk were used. The milk when received was mixed and divided into two lots of approximately 400 lb. each. One lot was used without heat treatment, whereas the second was either vat pasteurized, vacreated or pasteurized by the HTST method. The vat pasteurization procedure consisted of heating the milk to a temperature of 143–145° F. and holding for 30 minutes at that temperature, after which it was surface cooled to 35–40° F. The vacreation process consisted of preheating to a temperature of 145° F. for 5–10 minutes in a vat pasteurizer, after which the milk was run through a Vacreator at a temperature of 162–165° F., followed by cooling to 35–40° F. over a surface cooler. The HTST treatment consisted of heating the milk to 161–162° F. for 15–16 seconds followed by cooling in the regenerative and cooling sections of a plate pasteurizer.

Each series consisted of 12 split vats, the difference between the series being the type of pasteurization treatment employed. The pasteurized milk in each case was heated and held for periods sufficient to give a negative phosphatase test. Both raw and pasteurized lots of milk were held in a cold storage overnight, after which the milk was transferred to the cheese vats. Both batches were raised to the ripening temperature. An active starter then was added, ranging from 0.5 to 2 per cent for the pasteurized milk and 0.25 to 1.75 per cent for the unpasteurized milk. An average of 0.015 to 0.025 per cent acid was developed before setting in both cases.

CANADIAN CHEDDAR CHEESE

TABLE 1

Time, temperature and acidity schedules of experimental cheese from pasteurized milk

Operation	Time of operation	Time to next step		Temp.	Av. acidity
		(hr.)	(min.)		
Add starter	8:30	1	0	86	0.155
Setting	9:30	0	30	86	0.179
Cutting	10:00	0	15	86	0.118
Steam on	10:15	0	30	86	
Steam off	10:45	1	0	102	
Dipping	11:45	0	15	102	0.153
Cheddaring	12:00	3	0	102	0.175
Milling	3:00	0	15	94	0.511
Salting	3:15	0	30	90	
Hooping and pressing	3:45	0	30	86	
Dressing	4:15	Overnight			

The time, temperature and acidity schedules followed in making cheese from pasteurized milk are set forth in table 1. Table 2 compares the amounts of starter used and acidities at various stages for both the raw and pasteurized milk cheese. Wilson's (11) manufacturing procedure was followed for the pasteurized milk cheese. The raw milk cheese schedule was based on acid development at the different stages. Cheddaring was accomplished by piling the curd on both sides of the vat and letting it mat. After sufficient matting, the curd was cut into suitably sized slabs and turned every 10–15 minutes. The slabs were piled two and three high as cheddaring advanced, depending on the rapidity of the draining process. Completion of the cheddaring process was ascertained by the development of acid and a smooth, meaty texture of the matted curd. The milling and salting procedures were very similar to those used in the pasteurized milk.

The temperature of the curd at the time it was weighed and put into four Stilton-size hoops was 86° F. The hoops were placed in the press for 30 minutes and then taken out, the cheese properly dressed, replaced and left in the press overnight.

Two cheese of each lot of four were paraffined at a temperature of 220° F. for 10 seconds, whereas 2 of each lot were left unparaffined. This latter feature

TABLE 2

Comparisons of average percentages of starter used and acidities developed in the manufacture of Cheddar cheese from raw and pasteurized milk

Operation	Raw milk	Pasteurized milk
% starter used	0.89	1.48
Initial milk acidity (%)	0.175	0.155
Setting acidity (%)	0.193	0.179
Cutting acidity (%)	0.132	0.118
Dipping acidity (%)	0.178	0.153
Cheddaring acidity (%)	0.229	0.175
Milling acidity (%)	0.595	0.511

TABLE 3

Distribution of flavor scores for 80 cheese, from 12 split vats, raw and vat pasteurized, cured at 45 and 58° F., 48 paraffined and 32 unparaffined. Age = 3 mo.

Flavor score	No. of cheese in each classification							
	Raw				Vat pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
40 and over	0	0	0	0	5	4	6	5
39 to 39.9	5	3	4	1	7	7	2	3
38 to 38.9	4	6	3	6	0	1	0	0
35 to 37.9	3	3	1	1	0	0	0	0

was not included in the first four split vats. They then were placed in the curing room having a temperature of 58° F. until the first scoring period, after which one of each of the paraffined and unparaffined cheese was stored at 45° F., while the other pair was stored at 58° F.

TABLE 4

Distribution of flavor scores for 96 cheese, from 12 split vats, raw and vacreated, cured at 45 and 58° F., 48 paraffined and 48 unparaffined. Age = 3 mo.

Flavor score	No. of cheese in each classification							
	Raw				Vacreated			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
40 and over	1	0	1	0	6	4	6	3
39 to 39.9	7	5	6	5	6	8	6	8
38 to 38.9	3	4	4	5	0	0	0	1
35 to 37.9	1	3	1	2	0	0	0	0

All of the cheese were scored at ages of approximately 10 days, 3 months and 11 months. This was done by two, sometimes three, Dominion Dairy Produce Graders, without any knowledge on their part as to the treatment of the cheese except that they were experimental lots.

TABLE 5

Distribution of flavor scores for 96 cheese, from 12 split vats, raw and HTST pasteurized, cured at 45 and 55° F., paraffined and unparaffined. Age = 3 mo.

Flavor score	No. of cheese in each classification							
	Raw				HTST			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
40 and over	0	0	0	0	1	1	2	0
39 to 39.9	0	0	1	0	7	7	9	10
38 to 38.9	7	3	4	2	4	4	1	2
35 to 37.9	5	9	7	10	0	0	0	0

TABLE 6

Distribution of flavor scores for 372 cheese, from 36 split vats, all raw and all pasteurized combined, cured at 45 and 58° F., 144 paraffined and 128 unparaffined. Age = 3 mo.

Flavor score	No. of cheese in each classification							
	All raw				All pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
40 and over	1	0	1	0	12	9	14	8
39 to 39.9	12	8	11	6	20	22	17	21
38 to 38.9	14	11	11	13	4	5	1	3
35 to 37.9	9	17	9	13	0	0	0	0

RESULTS AND DISCUSSIONS

Scores at three ages were obtained and recorded. Since the data obtained were of necessity quite extensive, considerable condensing was necessary. The flavor score distributions for the 3-month old cheese, being representative of all, have been condensed and set forth in tables 3, 4, 5 and 6.

The Canadian scale of points for scoring cheese is as follows: Flavor-45 points, Texture-25 points, Closeness-15 points, Color-10 points, Finish-5 points.

Flavor and total score ranges for the various grades are as follows:

Grade of cheese	Flavor score range	Total score range
First	39 -- 45	92 and over
Second	37 and under 39	87 and under 92
Third	35 and under 37	85 and under 87
Below Third Grade	No score given	

That the use of pasteurized milk results in a more uniform and improved quality cheese has been adequately shown in tables 3, 4, 5 and 6. Table 7 com-

TABLE 7

Distribution of total scores for 210 cheese, from 36 split vats, raw and pasteurized, cured at 45 and 58° F., 128 paraffined and 112 unparaffined. Age = 3 mo.

Total score	No. of cheese in each classification							
	All raw				All pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
92 and over	14	10	13	9	27	26	28	27
87 to 92	17	12	14	17	5	6	0	1
85 to 87	1	10	1	2	0	0	0	0

pares the total scores of 36 pairs of raw and pasteurized lots of cheese revealing the predominant preference for the pasteurized milk cheese.

Differences in flavor scores between paraffined and unparaffined cheese of

TABLE 8

Average flavor scores at 10 days, 3 months and 11 months, for 80 cheese, from 12 split vats, raw and vat pasteurized, cured at 45 and 58° F., paraffined and unparaffined

Age	Raw				Vat pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
10 days	39.2	39.2	39.2	39.2	39.6	39.6	39.6	39.6
3 mo.	38.5	38.1	38.7	38.4	39.6	39.4	39.9	39.7
11 mo.	37.8	37.1	37.7	37.2	39.2	38.8	39.0	38.5

the raw and pasteurized groups are not very significant. However, the total score grade distribution shows a slight preference for the unparaffined lots.

Tables 8 through 11 portray the influence of the 3 heat treatments on the flavor of the cheese at 10 days, 3 months and 11 months.

The average flavor scores reveal a very distinct trend for the better in the pasteurized milk cheese. The older the cheese the greater the difference in

TABLE 9

Average flavor scores at 10 days, 3 months and 11 months, for 96 cheese, from 12 split vats, raw and vacreated, cured at 45 and 58° F., paraffined and unparaffined

Age	Raw				Vacreated			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
10 days	38.8	38.8	38.8	38.8	39.7	39.7	39.7	39.7
3 mo.	38.9	38.5	38.8	38.7	39.7	39.4	39.6	39.4
11 mo.	38.1	37.8	38.2	37.8	39.3	39.0	39.1	39.0

flavor score between raw and pasteurized, indicating better keeping quality of the pasteurized milk cheese. The average increases in flavor scores for the pasteurized milk cheese were 0.6 points at 10 days, 1.2 points at 3 months and 1.6 points at 11 months.

Expressed on a percentage grade distribution basis, the results indicate that pasteurization improved the flavor score considerably at both the 3 month and 11 month gradings, as shown in tables 12 and 13. Based on total scores, the

TABLE 10

Average flavor scores at 10 days, 3 months and 11 months, for 96 cheese, from 12 split vats, raw and high-temperature-short-time pasteurized, cured at 45 and 58° F., paraffined and unparaffined

Age	Raw				HTST			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
10 days	38.2	38.2	38.2	38.2	38.6	38.6	38.6	38.6
3 mo.	38.7	36.6	37.7	37.2	39.1	39.0	39.2	39.0
11 mo.	36.3	35.0	37.2	35.5	39.0	38.6	38.7	38.9

TABLE 11

Average flavor scores at 10 days, 3 months and 11 months, for 288 cheese, from 36 split vats, raw and pasteurized, cured at 45 and 58° F., paraffined and unparaffined

Age	All raw				All pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
10 days	38.7	38.7	38.7	38.7	39.3	39.3	39.3	39.3
3 mo.	38.7	37.7	38.4	38.1	39.4	39.3	39.6	39.4
11 mo.	37.4	36.7	37.7	36.8	39.2	38.8	38.9	38.8

percentage grade distribution at 3 months of age (table 14) also shows a marked preference for the pasteurized milk cheese.

On a percentage grade distribution basis, the first grade ratio of raw milk cheese to pasteurized milk cheese was 1:2 to 1:3 at 3 months of age. At 11 months this ratio was 1:4 to 1:6, depending on whether the cheese were par-

TABLE 12

Percentage grade distribution at 3 months, based on flavor score, for 288 cheese, from 36 split vats, raw and pasteurized, cured at 45 and 58° F., paraffined and unparaffined

Flavor grade	All raw				All pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
First	44.4	30.6	43.7	28.1	97.2	94.4	100.0	96.9
Second	52.8	47.2	46.9	62.5	2.8	5.6		3.1
Third	2.8	22.2	9.4	9.4				

affined or not, also the curing temperatures. Both uniformity and better quality can be achieved in Cheddar cheese made from pasteurized milk.

The raw milk cheese definitely did not stand up as well at 58 as at 45° F., which is quite logical. However, the difference between these two temperatures for the pasteurized batches was relatively small, indicating the possibility of utilizing a higher curing temperature to compensate, in part, for the delayed curing at 45° F. Curing temperatures of 58° F. or slightly higher are readily applicable to cheese made from properly pasteurized milk in which the flora is controlled.

TABLE 13

Percentage grade distribution at 11 months, based on flavor score, for 288 cheese, from 36 split vats, raw and pasteurized, cured at 45 and 58° F., paraffined and unparaffined

Flavor grade	All raw				All pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
First	22.2	13.9	21.9	12.5	86.1	75.0	81.2	84.4
Second	41.7	36.1	43.7	46.9	11.1	22.2	15.6	12.5
Third	36.1	50.0	34.4	40.6	2.8	2.8	3.1	3.1

The higher temperatures favor more rapid activity of the essential bacteria, thus hastening the curing process. The use of a curing temperature of 58° F. for raw milk cheese does not seem practicable because of the possibilities of off-flavor development.

One of the main criticisms of the use of pasteurized milk for making Cheddar cheese has been, and still is, the alleged lack of typical Cheddar flavor development. The authors have, on frequent occasions, examined some of the experimental cheese and found the pasteurized cheese to possess a good, clean Cheddar cheese flavor, more mild, mellow and uniform than that of the raw milk counterparts. The use of a higher (58° F.) curing temperature for pasteurized milk cheese will counteract the slow curing obtained at lower temperatures, yet will not forfeit the clean typical Cheddar flavor.

A preference study was made during a previous season using samples of cheese made from split vats of raw and pasteurized milk. These were obtained

TABLE 14

Percentage grade distribution at 3 months, based on total score, for 288 cheese, from 36 split vats, raw and pasteurized, cured at 45 and 58° F., paraffined and unparaffined

Total score grade	All raw				All pasteurized			
	Paraffined		Unparaffined		Paraffined		Unparaffined	
	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.	45° F.	58° F.
First	43.7	31.2	46.4	32.1	84.3	81.2	100.0	92.8
Second	53.1	37.5	50.0	60.7	15.7	18.8		7.2
Third	3.2	31.3	3.6	7.2				

from 8 pairs of cheese 10 months old. Samples were distributed among members of the University Staff, the Staff of the Dairy Branch of the Manitoba Department of Agriculture and that of the Winnipeg Office of the Marketing Service of the Dominion Department of Agriculture. Each recipient was asked to indicate his or her preference between the two samples which were labelled A and B. Of the 37 replies received, 20 preferred the pasteurized milk cheese, 15 preferred the raw milk cheese and 2 indicated no preference. This study, though carried out on a small scale, does reveal the preference tendency.

SUMMARY

1. The average flavor scores, of 288 cheese, made from raw and pasteurized milk, were in favor of the pasteurized milk cheese. The average flavor score increase at three separate gradings was 0.6 points at 10 days scoring, 1.2 points at 3 months scoring and 1.6 points at 11 months scoring.

2. The quality of the cheese, based on the flavor score obtained at 3 months of age, showed that 28.1-44.4 per cent of the raw milk cheese and 94.4-100 per cent of the pasteurized cheese were first grade. The percentage range depended on whether the cheese were paraffined or not and also on the curing temperatures employed. At 11 months of age, the percentages were 12.5-22.2 per cent

and 75.0-86.1 per cent, respectively, for the raw and pasteurized cheese. The balance of the samples in each case were of second grade quality or lower.

3. Of 37 preference reports received, 54 per cent favored pasteurized milk cheese, 6 per cent indicated no preference while 40 per cent preferred raw milk cheese.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. J. A. McManus, Dairy Produce Grader, in charge of the Winnipeg Office, Mr. H. S. Anderson, in charge of the Dairy Produce Inspection Service in Western Canada, and Mr. K. G. McKay, Senior Dairy Produce Grader for Western Canada, for scoring all of the experimental cheese; to Modern Dairies Limited, St. Boniface, Manitoba, for supplying milk pasteurized by the HTST method and to the employees of the Department of Dairy Science for their assistance in preparing the milk for the various experimental batches.

REFERENCES

- (1) BOWMAN, MAXWELL. An Epidemic of Typhoid Fever Due to Infected Cheese. *Can. Pub. Health J.*, **33**, 11: 541. 1942.
- (2) CAMPBELL, A. G., AND GIBBARD, J. The Survival of *E. typhosa* in Cheddar Cheese Manufactured from Infected Raw Milk. *Can. Pub. Health J.*, **35**, 4: 158-64. 1944.
- (3) EDITORIAL. Public Health Service Disease Outbreak Reports, 1945. *J. Milk Technol.*, **10**, 3: 125-127. 1947.
- (4) FABIAN, F. W. Cheese as the Cause of Epidemics. *J. Milk Technol.*, **9**, 3: 129-142. 1946.
- (5) GAUTHIER, J., AND FOLEY, A. R. A Cheese-Borne Epidemic of Typhoid Fever. *Can. Pub. Health J.*, **34**, 12: 543. 1943.
- (6) IRVINE, O. R., BRYANT, L. R., HILL, D. C. AND SPROULE, W. H. A Comparison of the Yield of Cheddar Cheese Obtained from Raw, Holder Pasteurized and HTST Pasteurized Milk. *J. Dairy Sci.*, **30**, 8: 530-31. 1947.
- (7) PRICE, W. V. Cheddar Cheese from Pasteurized Milk. *J. Dairy Sci.*, **10**: 155-68. 1927.
- (8) PRICE, W. V. Can Pasteurized Milk Cheese Compete with Raw Milk Cheese? *Natl. Butter Cheese J.*, **35**: 32-3. Nov. 1944.
- (9) TUCKEY, S. L. Making Cheese from Pasteurized Milk. *Natl. Butter Cheese J.*, **27**, 23: 22. 1936.
- (10) WALTER, H. E., AND LOCHRY, H. R. The Manufacture of Cheddar Cheese from Pasteurized Milk in Commercial Plants. *J. Dairy Sci.*, **28**, 8: 597-606. 1945.
- (11) WILSON, H. L. Making American Cheddar Cheese of Uniformly Good Quality. USDA Mimeo. BDIM-947. 1942.
- (12) WILSON, H. L., HALL, S. A., AND ROGERS, L. A. The Manufacture of Cheddar Cheese from Pasteurized Milk. *J. Dairy Sci.*, **28**, 3: 187-200. 1945.

STANDARDIZING HAY PALATABILITY TRIALS WITH CALVES

H. S. WILLARD

University of Wyoming, Laramie

The more palatable the hay, generally speaking, the greater is the consumption of it by calves. The problem of producing palatable hay is not confined to Wyoming, for this problem has no geographical boundaries. In Wyoming, however, the farmer's grain supply usually is limited, and he is more likely to have an ample supply of hay. The quality of the hay, consequently, is of great importance in the proper development of young stock in this state, because of the large place this feed occupies in the calf ration.

There is no known chemical method of testing for palatability of hay. There are some chemical constituents which, by their presence or absence, affect palatability, among them protein and sugar, but the amount of these present is not always directly correlated with how well cattle like the feed (1). Chemical methods, for example, cannot measure physical characteristics, such as brittleness of stems. Measurement of palatability of hay must be accomplished by feeding it to the class of livestock for which it is intended. The accuracy of this method depends upon the repeatability of results. Variability of consumption of the same hay, as determined by experimental method, must be controlled so that the various factors influencing palatability, such as type of soil, time of cutting and method of curing, may be studied during several growing seasons with the assurance that the methods used will give comparable results from one season to another.

The author, in this paper, has confined himself to experiments in devising a method for determining palatability of hay fed to calves. The disclosure of certain factors influencing palatability, such as variety or time of cutting, is purely incidental to the main objective.

A number of years ago, the author determined that hay allowance, within certain limits, made a considerable amount of difference in the amount of hay consumed by calves when other factors were constant (2). Hay consumption followed the hay allowance closely; when hay allowance went up, hay consumption went up.

Several factors in this experiment made it difficult to measure the exact effect of hay allowance on hay consumption. For one thing, as the calves grew older, the grain allowance was changed so that the total ration would conform with the Morrison Standard for total digestible nutrients. This meant that the grain allowance was lowered as the hay allowance was increased, so that it was difficult to ferret out which factor influenced consumption the more. Then too, it was shown that when hay consumption was calculated per 100 lb. live weight, hay consumption per hundredweight increased as the calves grew older. Here again, a question arose as to whether the increase in capacity and appetite or the increase in hay allowance caused an increase in hay consumption.

Received for publication July 28, 1948.

EXPERIMENTAL PROCEDURE

In an attempt to answer some of these questions, three types of hay (table 1) were fed to calves ranging in age from 3 to 12 months. Altogether, 34 head of Holstein females were fed in individual box stalls so that daily hay refusals could be ascertained.

The calves were given a grain mixture which consisted of three parts ground barley, three parts ground oats and one part wheat bran. One per cent common salt was added to this mixture. The grain allowance depended to some extent upon the calf's weight and age. When a calf was underweight, more grain was fed in an attempt to bring the calf back to normal weight. The milk allowance varied, in some cases being fed only until the calf reached 60 days of age, in other cases for periods of up to 150 days of age. Both whole and skim milk were included in the rations. The amount of milk fed was reflected in the calf weights, the calves

TABLE 1
The chemical analysis of the three types of hay

	<i>H O</i>	<i>E.E.</i>	<i>Fiber</i>	<i>Crude Pro</i>	<i>N.F.E.</i>	<i>Ca</i>	<i>P</i>
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Alfalfa, second cutting, good quality	10.0	2.65	21.69	17.29	40.48	1.46	0.22
Alfalfa, second cutting, poor quality	11.8	1.53	30.68	13.60	35.39	1.56	0.18
Native hay from the Laramie Plains	10.0	2.14	31.90	7.10	43.05	0.29	0.14

getting smaller amounts of milk or getting it for shorter periods of time usually being under normal weight at the time they were weaned.

The feed allowed and consumed was plotted against age and weight of each individual calf in order to secure the hay fed and consumed at definite weights and ages. The feed allowed and consumed then was interpolated at 10 lb. and 10 day intervals. Charts were made using the mean of each group of calves.

RESULTS

Weight, age, and hay quality affect results. Figures 1 and 3 show the relationship between the live weight of the calves and their hay consumption, as well as the influence of the amount of hay placed before the calf. Figures 2 and 4 show the relationship between the age of the calves and their hay consumption, plus the influence of the amount of hay offered.

An attempt was made in this feeding experiment, as was mentioned earlier, to correct the ration for weight and age. There also was a correction for the difference in quality of hay. The less palatable alfalfa was of a poorer feeding quality, and accordingly, more was offered to compensate for the feeding deficiency. Likewise, some attempt was made to correct the somewhat slower growth of the calves getting low grade alfalfa by adding more grain to the ration when the animals lagged in growth.

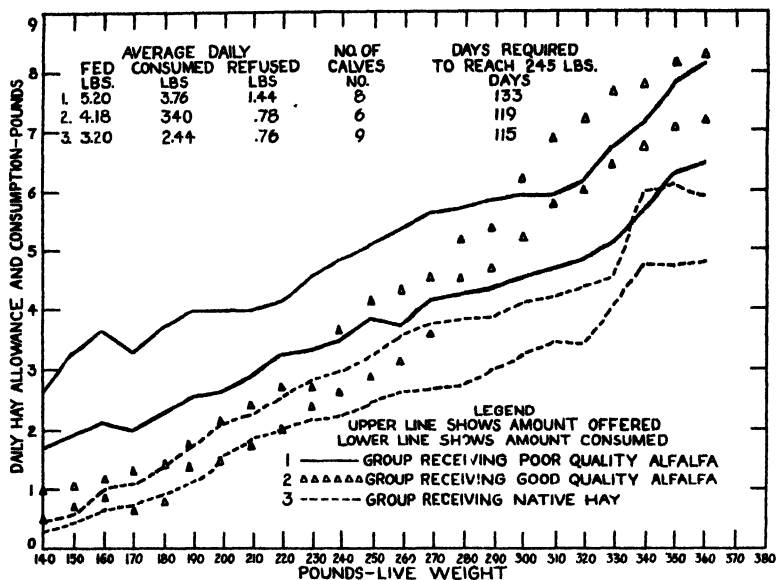


FIG. 1. Consumption of hay as affected by amounts offered to smaller calves.

These discrepancies are noticeable in figures 1 and 2, and again in figures 3 and 4. In figure 1, the calves receiving good quality alfalfa surged ahead of the other two groups in weight for age. Figures 2 and 4 show that after the calves passed 140 days of age, they were able to utilize more hay under the

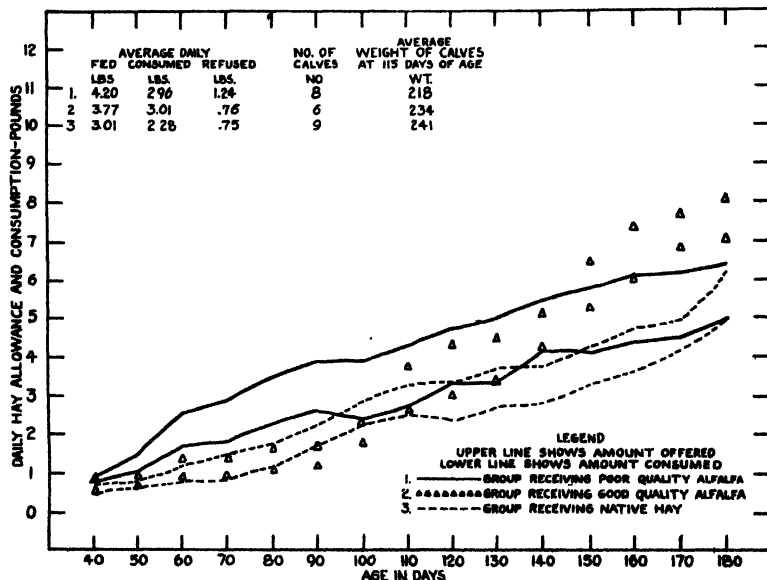


FIG. 2. Consumption of hay as affected by amounts offered to younger calves.

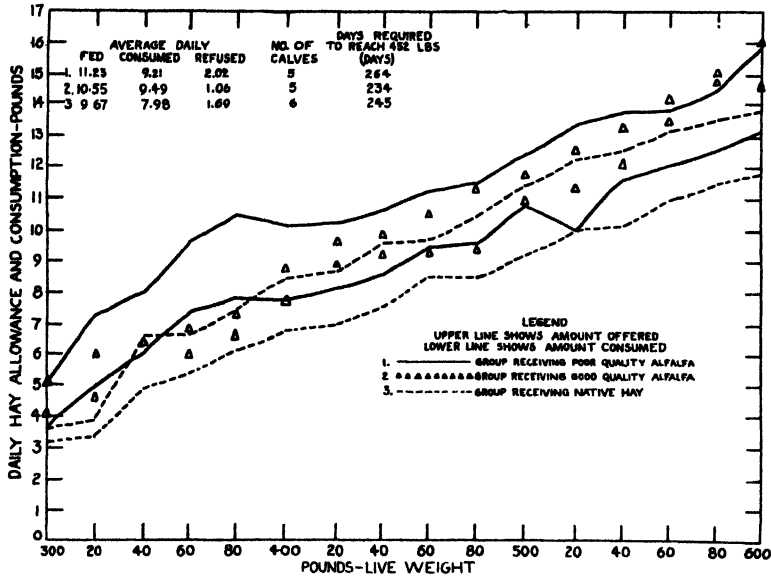


Fig. 3. Consumption of hay as affected by amounts offered to heavier calves.

ration provided for them, because of heavier weight for their age. At the same time, since the calves getting poorer quality hay were somewhat slower in reaching the 300 lb. level, and thus were somewhat older, the hay allowance was increased (fig. 3).

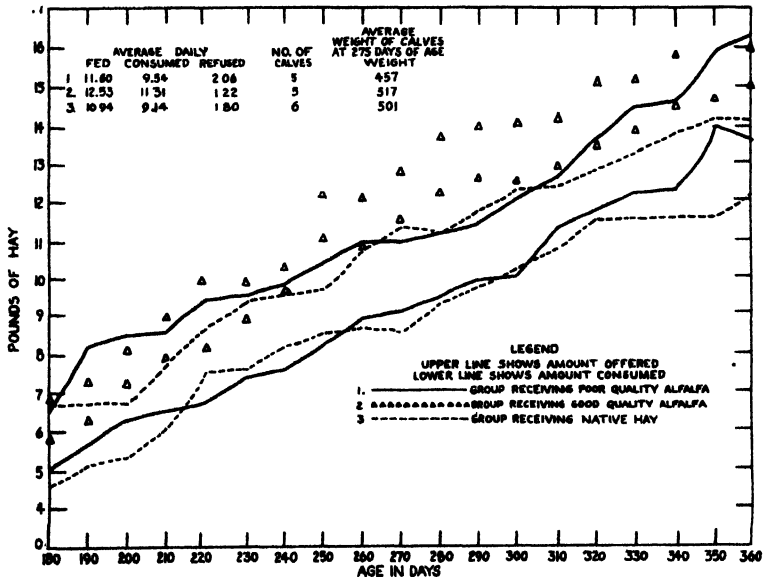


Fig. 4. Consumption of hay as affected by amounts offered to older calves.

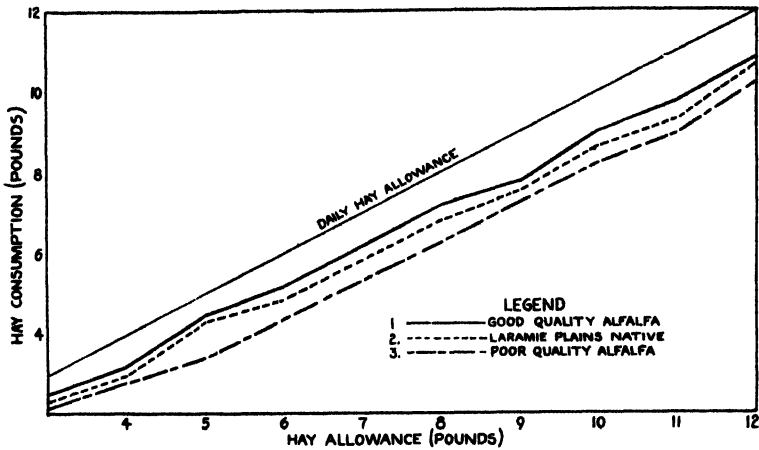


FIG. 5. Daily hay consumption of equal allowance basis.

All four figures, in general, illustrate the many variable factors which affect any measure of palatability. These factors are extremely difficult to control and to standardize. For example, it is theoretically possible to get three calves which will gain at an approximately equal rate. But if the calves are fed three different kinds of hay, all of equal feeding value but of varying palatability, the rate of gain in each of the calves will be affected by the palatability of the hay eaten, since the calf will reject more of the unpalatable hay. So, although

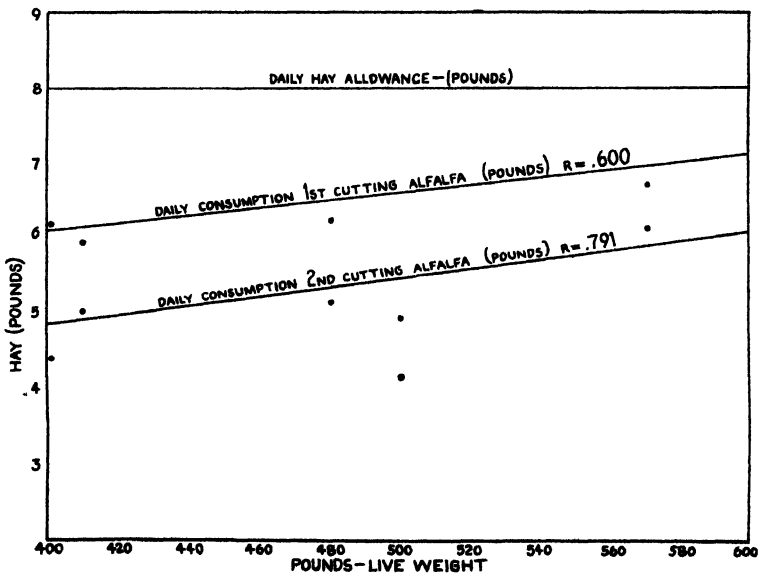


FIG. 6. Consumption of first and second cuttings of alfalfa, as affected by live weights of calves.

the three calves may start out on a relatively uniform basis, by the time they reach 6 months of age, they are no longer strictly comparable. The palatability results likewise are not strictly comparable.

Figures 1, 2, 3 and 4 give only a rough approximation of the palatability of the three different hays. The gap between the amount offered and the amount consumed suggests in a small degree whether or not the hay is palatable, but there is no real basis for comparison of the hays at any one point on the diagram.

Standardized amount of hay offered. Figure 5 compares the three hays entirely on a basis of how much hay was allowed and how much was rejected. The allowance for each calf was adjusted according to the animal's weight and age and took into consideration the amount of milk and grain which was given to the animal. This simply means that if the calf's ration required 7 lb. of hay in order to keep the ration up to the Morrison Standard for total digestible nutrients, the animal's other dietary needs theoretically were being satisfied, and its rejection or acceptance of the 7 lb. of hay would constitute about as accurate a measure of the hay's palatability as it would be possible to get.

The hay allowance here is a constant figure, while the variables of age and weight to some extent offset each other and are adjusted for in the ration which is fed to the calf. There are other variables which will be indicated in figure 6, but in general, with this procedure, where a fixed amount of hay is fed, one can expect a fairly uniform hay consumption from year to year. Thus, with a uniform and comparable consumption of hay set against a measured hay allowance, the rejection and consumption of the hay provide a fixed standard against which the palatability of any hay can be measured over a period of several years.

Using the consumption of each individual calf, the coefficient of variation of the consumption of these three hays at different hay allowances ranged from 1 to 3 per cent. The standard deviation ranged from 0.4 to 0.8 and the standard error between 0.05 and 0.2. The pooled variance was 0.37. The mean number of observations of daily consumptions for each hay allowance was 33, 22 and 38 for the good alfalfa, poor alfalfa and native hay, respectively. The mean number of calves for each of the above hays was 15, 8 and 11, respectively. The mean weight and age of the calves steadily increased with each raise in hay allowance, but varied 100 lb. in weight and 2 months in age within each grouping. The amount of grain consumed by the calves within each grouping varied as much as 2 lb. per head daily.

Other variable factors. There may be many factors other than hay allowances which should be controlled in hay palatability trials. From the available records in this experiment, there were indications of significant individual variations in hay consumption. There also is a progressive increase in hay consumption per lb. of live weight as the calf grows and as it becomes older.

To test this point more fully and also to determine the effect of live weight on consumption, both first and second cutting alfalfa hay were fed in equal quantities for 10 days to six calves of different weights. Figure 6 shows the result. For calves varying in weight from 400 to 600 lb., there was an upward

trend in consumption. The correlation between weight and consumption falls short of significance for such a small number of animals, but is statistically significant when the 500 lb. calf is eliminated. The 500 lb. calf apparently is the shy hay consumer in both instances. The results of the test indicate that one may well eliminate from palatability tests calves which are low hay consumers.

DISCUSSION

From the results of these experiments, certain rules can be formulated for determining relative palatability of hay for calves with the assurance of fairly uniform results year after year. The calves should be given a definite hay allowance according to age and weight. The data show that relative palatability of the three hays, when based upon consumption of the hay, depends upon the amount of hay offered. The relative palatability should be measured by consumption when equal amounts are fed. The error on repeated tests at different weights of calves was very small. By using this constant as a base (definite consumption of the same hay at definite allowance levels), one may not only compare the palatability of several types of hay grown during the same season but be assured of comparable results throughout many growing seasons. A feeding standard should be as a guide for determining the proper grain allowance. As shown by the small error in consumption in repeated tests, 1 or 2 lb. of grain, more or less, to make the ration conform to the Standard, makes little difference in hay consumption. Calves which are noticeably low hay consumers should be eliminated from palatability experiments.

Figure 5 shows clearly the difference in the relative palatability of the three hays and simplifies the confusion which exists in figures 1, 2, 3 and 4. The experimental error at any point along the consumption lines of each hay is very small, even though the calves varied in weight and age. The method of presentation used in figure 5 has the additional advantage of accumulating comparable data on the same chart over a period of years.

SUMMARY AND CONCLUSIONS

A study of the hay consumption of 34 Holstein calves from 3 to 12 months of age showed that consumption followed hay allowance over a wide range of weight and age. When the hay allowance was increased by 1 lb. daily, the coefficient of variation in consumption of three types of hay ranged from 1 to 3 per cent, the standard deviation ranged from 0.4 to 0.8, and the standard error between 0.5 and 0.2. Because of this small variation, hay palatability trials may be standardized to give comparable results over a period of several years by simply comparing hay consumption with a set hay allowance even though there may be a variation in weight for age and in grain consumption.

REFERENCES

- (1) WILLARD, H. S., Factors Influencing the Palatability of Hay. Wyo. Agr. Expt. Sta. Bull. 199. 1933.
- (2) WILLARD, H. S., Hay Consumption of Holstein Calves. J. Dairy Sci., 21 (3): 153-160. 1938.

TOTAL MILK PRODUCTION AS AFFECTED BY TIME OF MILKING AFTER APPLICATION OF A CONDITIONED STIMULUS¹

GERALD M. WARD AND VEARL R. SMITH

Department of Dairy Husbandry, University of Wisconsin, Madison

It generally is believed that the most efficient practical method of milking is stimulation of the udder and teats, followed shortly by rapid milking. Babcock (1) reported in 1889 the effect of milking the quarters of the udder one at a time in different order. The quarters milked second produced the greatest amount, and the production of quarters milked first, third and fourth were in descending order. Experiments of a similar design which essentially substantiated the results obtained by Babcock later were conducted by Beach (2), Emery (8) and Plumb (12). Bitting (3) and Miller and Petersen (10) repeated Babcock's work, but found that milk production decreased from the first to the fourth quarter milked. Babcock (1), Crowther (4) and Skrodel (13) demonstrated reduced milk production when the milking act was prolonged unduly. Miller and Petersen (10) also obtained a reduced milk flow by stimulation of cows 20 minutes before milking. Dahlberg (5) stated that reduction of milking time from about 10 minutes to 4 or 5 minutes resulted in greater annual production. However, Dodd and Foot (6) obtained a slight reduction in total milk yield by reducing the milking time from a mean of 8 to 5 minutes. Knodt *et al.* (9) found no significant differences in total milk production of cows milked at intervals of 2, 4, 6, 8 and 10 minutes after preparation for periods of 35 and 70 days, nor in another experiment of 90 days where the cows were milked at 2, 5, 10 and 20 minutes after preparation.

Ely and Petersen (7) state that the expulsion of milk from the alveoli into the ducts is brought about by the action of a posterior pituitary factor (or factors) which is discharged as the result of conditioned stimuli which precede or the mechanical stimulations which accompany milking, or both. According to Pavlov (11), a reflex can be conditioned by the close association of a heretofore neutral stimulus to an established reflex. Where varying amounts of time are allowed to elapse between the conditioned stimulus and the subsequent unconditioned stimulus, the conditioned reflex is known as a delayed reflex. If a conditioned reflex is to be kept at a constant strength, the conditioned and unconditioned stimuli must be applied regularly or the conditioned reflex gradually weakens and becomes extinct. Delayed reflexes become extinct more readily than simultaneous reflexes. In the modern methods of milking, where the udder is washed and massaged shortly before the attachment of the teat cups, the heretofore neutral stimuli, *i.e.*, hot water and massage, become conditioned stimuli.

The object of these investigations was to ascertain the duration of effectiveness

Received for Publication July 31, 1948.

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

TABLE 1
Effect on total milk production of milking at various intervals after stimulation

No. of cow	296				310				311				359				874			
Half of udder	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left		
Period	T.A.S.*	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.	Milk T.A.S.		
	(min.)	(lb.)	(min.)	(lb.)	(min.)	(lb.)	(min.)	(lb.)	(min.)	(lb.)	(min.)	(lb.)	(min.)	(lb.)	(min.)	(lb.)	(min.)	(lb.)		
Preliminary	2	7.5	2	6.6	2	12.1	2	11.8	2	9.6	2	8.7	2	13.2	2	13.5	2	11.5	2	10.7
I	2	7.2	8	6.1	2	11.8	4	12.0	2	8.9	12	7.4	2	12.6	16	9.4	2	11.3	20	9.3
II	16	6.6	2	6.3	12	8.5	2	11.5	20	8.1	2	8.1	4	12.4	2	10.8	8	11.3	2	9.8
III	2	6.8	12	5.4	2	10.1	8	9.2	2	7.9	16	6.8	2	11.5	20	7.4	2	10.8	4	9.4
IV	20	5.9	2	5.5	16	7.7	2	10.2	4	7.7	2	7.1	8	10.7	2	9.2	12	9.6	2	8.9
V	2	6.4	4	5.3	2	8.8	20	6.0	2	7.6	8	6.4	2	10.6	12	6.5	2	10.3	16	7.6
Post	2	6.3	2	5.3	2	9.3	2	8.9	2	7.2	2	6.7	2	10.4	2	7.4	2	10.3	2	8.0

* T.A.S. = Time after stimulation.

of a conditioned stimulation, i.e., a hot water wash and massage of the udder and teats, on the discharge of milk from the mammary gland.

EXPERIMENTAL PROCEDURE

Five cows, four Holsteins and one Brown Swiss, were selected with as much variation in production and stage of lactation as possible. The mean production of each half of the udder was determined during a 10-day preliminary period by milking each half of the udder simultaneously into a separate container. Each cow in the experiment had been conditioned to a hot water wash and massage several minutes before milking since the beginning of their respective lactation periods. During the preliminary period, the cows were prepared for milking by a hot water wash and massage, and the milkers were attached 2 minutes after this stimulation. The cows were prepared in the same manner during the experimental periods. However, during the experimental periods, one half of the udder, which served as a control, was milked at 2 and the other half at 4, 8, 12, 16 or 20 minutes after stimulation. One half of the udder alternately served as control and experimental. The experiment was of Latin Square design. Within an experimental period, each of the five treatments was assigned to a different cow, and in the course of the five experimental periods each cow was subjected to every treatment. Treatments were at random. The experimental periods were of 5-day duration, followed by a 2-day transition period. The final experimental period was followed by a 5-day post-experimental period.

The basis for evaluating the effect of the treatments was the average production of the cows milked after 2-minute intervals immediately preceding and following an experimental period. This measure took into consideration the decline in lactation.

RESULTS

The production of the cows during the period of experimentation and the design of the experiment are presented in table 1. Each half of the udder served as its own control. The analysis of variance of the data is presented in table 2. The least significant difference at the 1 per cent level was found to be 6.50 lb. of milk for the treatment total. Consequently, the decrease in milk production,

TABLE 2
Differences in average milk production of experimental and control periods

Cows	Differences in average milk production as compared to average base production when milking followed stimulation by:				
	4 min.	8 min.	12 min.	16 min.	20 min.
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
296	- 0.10	- 0.35	- 0.50	- 0.40	- 0.70
310	+ 0.35	- 1.65	- 2.45	- 1.75	- 3.55
311	- 0.05	- 0.50	- 1.00	- 0.80	- 0.30
359	+ 0.35	- 0.35	- 1.80	- 2.75	- 2.60
874	+ 0.05	+ 0.25	- 0.95	- 0.85	- 0.95
Total	+ 0.60	- 2.60	- 6.70 ^a	6.55 ^a	8.10 ^a

^a Significant at the 1 per cent level.

when the cows were milked at 12, 16 and 20 minutes after stimulation, was highly significant. The differences in production obtained by milking at 2, 4 and 8 minutes after stimulation were not significant. The decrease in production which occurred at the 8 minute interval was not significant with the numbers involved.

DISCUSSION

The time required to milk cows a quarter at a time was not given by Babcock (1), Beach (2), Bitting (3), Emery (8) and Plumb (12), but Miller and Petersen (10) state that about 15 and 20 minutes elapsed before the milking of the third and fourth quarters, respectively. A statistical analysis of Babcock's data shows no significant difference in production for the first three quarters milked. Our results confirm the findings of Miller and Petersen (10). However, our results are not in agreement with the work reported by Knodt *et al.* (9). This discrepancy may be explained by their cows becoming unconditioned to the stimulation because of too long an interval between the application of the conditioned stimulation and the unconditioned reflex over periods of 35, 70 and 90 days. The failure of Dodd and Foot (6) to obtain a definite increase in production by decreasing the milking time is consistent with the results of this experiment, because the mean time of their milking period at the start was only a little over 8 minutes. Furthermore, the purpose of their experiment was to train cows for more rapid milking, and at times the cows were not completely milked out. They obtained a lower fat test which perhaps was a result of incomplete milking.

Four of the five cows showed a decrease in production when milking began 8 minutes after stimulation had been applied. Although statistical analyses of these data show this decrease in production to be insignificant, the decrease indicates that 8 minutes is near the threshold level. The results of these experiments would indicate that in general practice, milking should begin before 8 minutes have elapsed after a cow has been stimulated to discharge her milk if maximum production is to be obtained.

SUMMARY

An experiment was conducted to determine whether there was any difference in production when cows were milked at 4, 8, 12, 16 and 20 minutes after a conditioned stimulation, as compared with 2 minutes after stimulation.

There was a highly significant decrease in production when cows were milked 12 or more minutes after a conditioned stimulation.

The authors express their appreciation to Dr. J. E. Torrie for the advice given in the planning of this experiment and the statistical analyses of these data.

REFERENCES

- (1) BABCOCK, S. M. Variations in the yield and quality of milk. Sixth Annual Rept. Wis. Agr. Expt. Sta., pp. 42-62. 1889.
- (2) BEACH, C. L. The yield of quarters on same side of udder. Conn. (Storrs) Agr. Expt. Sta. Rept., pp. 135-137. 1904.

- (3) BITTING, A. W. The physiology of milk secretion. 14th Annual Rept. Ind. Agr. Expt. Sta., pp. 51-88. 1901.
- (4) CROWTHER, C. The significance of the act of milking. Brit. Assoc. Advancement Sci. Rept. 85th Mtg., pp. 779-780. 1915.
- (5) DAHLBERG, A. C. The influence on milk production of a definite time interval for milking cows by machine. N. Y. State Agr. Expt. Sta. Bull. 697. 1941.
- (6) DODD, F. H., AND FOOT, A. S. Experiments on milking technique. 1. Effect of washing the udder with hot water. 2. Effect of reducing milking time. J. Dairy Research, 15: 1-17. 1947.
- (7) ELY, F., AND PETERSEN, W. E. Factors involved in the ejection of milk. J. Dairy Sci., 24: 211-224. 1941.
- (8) EMERY, F. E. Milk records and tests. N. C. Agr. Expt. Sta. Bull. 116. 1895.
- (9) KNOTT, C. B., REID, J. J., WILLIAMS, P. S., AND KESLER, F. M. The effect of the interval between washing of the udder and attachment of milking machines upon the bacterial flora and milk production of dairy cows. J. Dairy Sci., 30: 580-581. 1947.
- (10) MILLER, K., AND PETERSEN, W. E. Some factors involved in efficient milking. J. Dairy Sci., 24: 225-238. 1941.
- (11) PAVLOV, I. P. Lectures on conditional reflexes. Translation by Gnatt and Volbroth. International Publishers, New York. 1928.
- (12) PLUMB, C. S. The udder of the cow. Ind. Agr. Expt. Sta. Bull. 62. 1896.
- (13) SKRODEL, L. Der Einfluss der Milchgeschwindigkeit auf die Sekretion der Milch und des MilCHFettes. Milch. Forsch., 19: 95-105. 1937.

EFFECT OF LACTATION AND RATION ON THE FAT AND VITAMIN A LEVEL OF SOW'S MILK¹

J. P. BOWLAND, R. H. GRUMMER, P. H. PHILLIPS, AND G. BOHSTEDT

*From the Departments of Animal Husbandry and Biochemistry,
University of Wisconsin, Madison*

The composition of sow's milk has been studied less than that of milk of the other domestic animals. Several studies have been reported on the fat and vitamin A content of sow's milk, but most of these have been quite limited in scope. Reports from the Wisconsin Agricultural Experiment Station as early as 1897 stated that the fat content of normal milk from sows averaged 7.06 per cent. This study by Henry and Woll (9) was on only seven samples of milk. Later Woll (17) reported a mean fat percentage of 5.97 for five samples of sow's milk. Davies (6), in reporting on the composition of milk from various species of mammals, gave values ranging from 4.55 to 9.54 per cent fat for porcine milk.

Hughes and Hart (10) reviewed the early literature on the gross composition of sow's milk. From the data of other workers, they calculated combined results of 6.9 per cent fat. In their own work with two sows, they obtained a mean value of 5.3 per cent fat for normal milk and 5.1 per cent fat for colostrum.

Willett and Maruyama (16) reported that the percentage of fat in sow's milk increased with the fat content of the ration. The average percentage of fat in the milk of sows on dry concentrates only was 6.1, and for sows on garbage only was 9.6 per cent. They also observed an increase in fat content of the milk with an advance in the stage of lactation. Braude *et al.* (4) showed conversely that after the rapid change from colostrum to milk, the fat content decreased appreciably as the lactation advanced.

Mean values for 18 samples of sow's colostrum of 3.4 per cent fat and 347 I.U. of vitamin A per 100 ml. of colostrum are reported by Braude *et al.* (5). In a later paper (4), they reported the following values for colostrum: 4.05 ± 0.43 g. of fat per 100 g. of milk, and 71.1 ± 9.1 I.U. vitamin A per g. fat; and for normal milk: 8.17 ± 0.17 g. fat per 100 g. of milk, and 11.0 ± 0.4 I.U. vitamin A per g. fat.

The study reported by Braude *et al.* (4) is the only work in the literature where the vitamin A content of the milk is calculated on the basis of the fat rather than the total milk volume. However, several other workers have reported on the vitamin A content of sow's milk. Benham (1) found that the vitamin A content of sow's colostrum ranged from 131 to 254 I.U. per 100 ml. on the first day. Work reported by Schofield *et al.* (12) showed that giving sows large doses of vitamin A late in pregnancy increased the concentration of this vitamin in the colostrum.

Received for publication August 5, 1948.

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station, Madison.

EXPERIMENTAL PROCEDURE

The data presented in this paper are the results of analyses for fat and vitamin A levels of the milk from ten sows carried through gestation on pasture, and ten sows carried through gestation in dry lot. The sows on pasture were self-fed a grain ration supplemented with animal protein, while those in dry lot were self-fed a grain ration supplemented with plant protein and 5 per cent alfalfa meal. The sows on pasture were five Poland Chinas and five Chester Whites, while all those in dry lot were Poland Chinas. All sows were in their

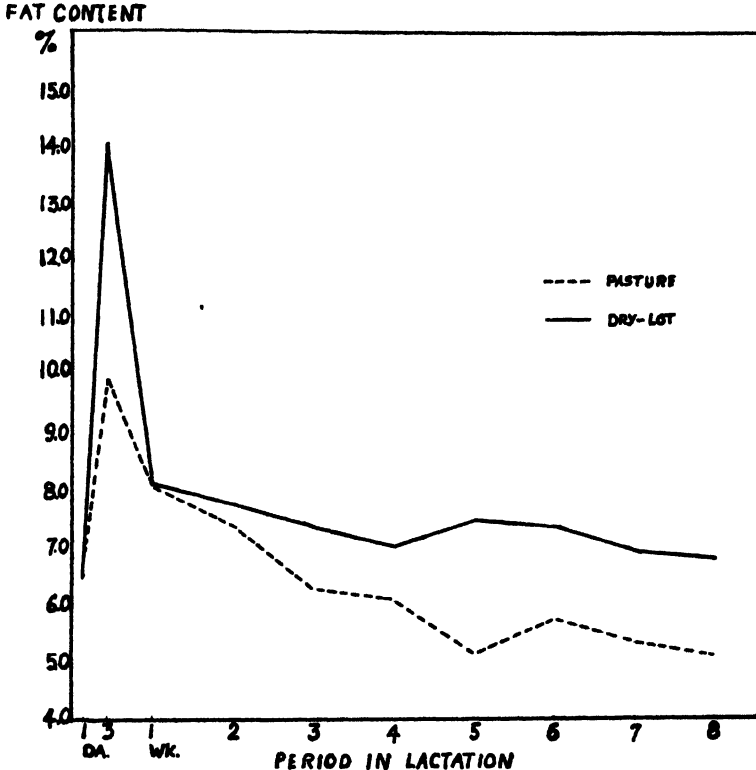


Fig. 1. The fat content of milk from pasture and dry-lot sows.

first or second lactation period. The farrowing dates of the sows in the pasture group ranged between September 24 and October 11, 1947, and the sows in the dry-lot group farrowed between November 12 and December 26, 1947. They weaned an average of 7.1 and 4.8 pigs, respectively.

The sows were milked on the day of parturition as soon after farrowing as possible, then on the third day and at the end of the first week and each subsequent week thereafter until weaning the litters at 8 weeks of age. The let-down of milk was induced by the injection of Pitocin (Parke, Davis and Co.) as described by Bowland *et al.* (2) and first used in cows by Ely and Petersen (7).

The determination of vitamin A in the 201 samples was run according to a slight modification of the method of Boyer *et al.* (3). The 195 fat determinations were made with the use of the Minnesota Babcock reagent. Eleven samples were run by the official Mojonnier Fat Extraction method to check on the accuracy of the Minnesota Babcock method. In all 11 cases, the Mojonnier method gave higher readings than the Minnesota Babcock method, the mean difference being 0.69 per cent fat. Because of this consistent difference, a logical inference might be that sow's milk contained a high phospholipid con-

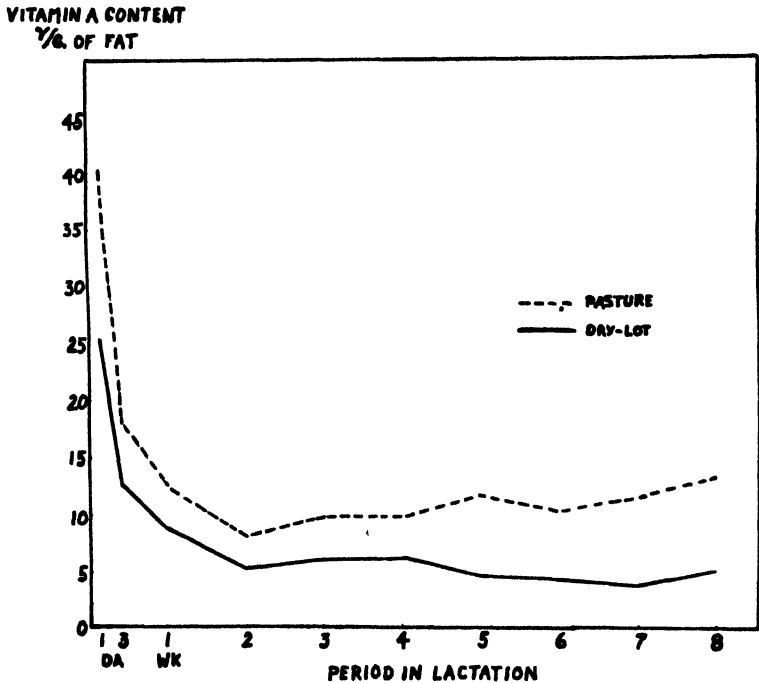


FIG. 2. The vitamin A content of milk from pasture and dry-lot sows.

tent, as the ether extraction would remove the phospholipids, whereas the Minnesota Babcock reagent would not.

RESULTS

The mean results of the fat determinations throughout lactation are shown in figure 1, and results of the vitamin A determinations calculated on the basis of fat are shown in figure 2. The average fat and vitamin A content in the colostrum and normal milk of each individual sow on experiment is shown in table 1.

The fat content of the normal milk from the dry-lot sows was consistently higher than that from pasture sows. However, in the case of first-day colostrum, the colostrum of the pasture sows had an average fat content of 6.73 per cent

and that of the dry-lot sows 6.45 per cent. Very probably this lower fat content of milk from sows previously on pasture was because of an increased volume of milk produced, as they weaned more pigs than the dry-lot group.

No breed difference was noted in the pasture sows where both Chester White and Poland China breeds were on experiment. The average fat content of the normal milk was 6.07 per cent for Chester Whites, and 6.17 per cent for Poland Chinas. There was no difference in lactational trend between the two breeds. The milk of both breeds in the pasture group, as well as the dry-lot group,

TABLE 1

The mean fat and vitamin A content of the milk of individual sows

Group	Breed	Fat content			Vitamin A content		
		Colostrum		Normal milk	Colostrum		Normal milk
		1st day	3rd day	Av. 1-8 wk.	1st day	3rd day	Av. 1-8 wk.
		(%)	(%)	(%)	(γ /g. of fat)	(γ /g. of fat)	(γ /g. of fat)
Pasture	C.W.	8.1	10.9	5.07	22.71	6.24	12.19
	C.W.	6.8	9.6	5.35	18.94	15.86	11.49
	C.W.	7.1	12.8	6.10	18.98	12.60	12.33
	C.W.	7.2	7.9	6.34	22.22	47.20	13.35
	C.W.	9.5	10.0	7.49	21.93	8.32	8.90
	P.C.	6.7	8.8	5.50	10.75	21.47	12.63
	P.C.	4.9		5.73	36.53		10.49
	P.C.	1.4	12.5	6.09	201.66	18.07	9.46
	P.C.	8.9		6.75	9.82		8.14
	P.C.		7.5	6.78		15.30	10.36
	Av.	6.73	9.99	6.12	40.23	18.13	10.93
Dry-lot	P.C.	7.1	11.3	6.15	20.38	18.09	6.46
	P.C.	4.7	11.5	6.79	27.13	29.08	6.69
	P.C.	7.4	13.5	6.90	12.53	8.93	6.29
	P.C.	5.0	11.0	6.95	34.96	10.75	5.22
	P.C.	4.1	8.4	7.01	42.98	14.07	5.04
	P.C.	10.9	19.8	7.45	10.95	9.91	4.65
	P.C.	5.0	15.6	7.54	36.93	6.28	6.08
	P.C.	7.4	15.7	8.39	15.97	8.10	4.57
	P.C.	9.4	16.4	8.58	18.46	10.00	4.83
	P.C.	3.5	17.2	8.73	33.77	10.25	7.02
	Av.	6.45	14.04	7.45	25.41	12.55	5.69

showed a tendency to decline in fat content as lactation advanced up to weaning at 8 weeks of age. This observation agrees with Braude *et al.* (4) but disagrees with Willett and Maruyama (16).

The milk fat from the pasture sows showed a considerably higher concentration of vitamin A than the milk fat from dry-lot sows. This difference was wider when calculated on the basis of fat than on the basis of volume of milk because of the inverse relation of the fat content of the milk in the two groups. As lactation advanced the spread between the vitamin A concentration of the milk from the two groups became wider as shown in figure 2.

The vitamin A concentration in the fat was down in the range of normal milk, namely 12.51 γ per g. of fat for pasture sows and 8.94 γ per g. of fat for dry-lot sows at the end of the first week of lactation. The vitamin A content of normal milk fat was about one fifth of that in the colostrum fat in the dry-lot sows, and one quarter in the pasture sows. There was a rapid drop in vitamin A the first 3 days of lactation, from 25.41 to 12.55 γ per g. fat in the dry-lot sows, and from 40.23 to 18.13 γ per g. fat in the pasture sows. Except for one sow in the pasture group, the first day vitamin A did not vary appreciably between the two groups. The drop in vitamin A concentration in the first 3 days did not show up when the vitamin A was reported on the basis of 100 ml. of milk as shown by Bowland *et al.* (2). However, the sudden unexplained rapid increase in fat in these first 3 days caused the concentration of vitamin A per g. of fat to be lowered.

The vitamin A content of the fat in the milk of the pasture group showed a gradual increase in advanced lactation, whereas the dry-lot group tended to decline. There generally was about a three- to fourfold variation in the vitamin A content in the colostrum and normal milk within groups.

DISCUSSION

The fat content of colostrum milk obtained in these studies was considerably higher than the values of 4.05 and 3.4 per cent reported by Braude *et al.* (4, 5), respectively. No definite change could be noted between the fat content of colostrum obtained before the pigs suckled and that obtained shortly afterward. There are no reports in the literature of high values similar to those obtained in this work for fat content of colostrum on the third day of lactation. Braude *et al.* (4) showed a very rapid rise in the first few days but not to the same extent.

The milk fat values of 7.45 and 6.12 per cent for dry-lot and pasture sows, respectively, were lower than the values obtained by Braude *et al.* (4) but similar to the combined calculated values of other workers reported by Hughes and Hart (10). This variation would be expected, as the fat content of sow's milk seemed to be influenced very easily as illustrated by Willett and Maruyama (16), who obtained a rise from 6.1 to 9.6 per cent fat by feeding a high-fat diet. Reece (11) reported that factors known to influence variation in fat content of cow's milk are breed and individuality of the cow, state of lactation, season of the year, feed, age of animal, day to day variation, time of milking, frequency of milking and the first-and last-drawn milk. In this work, individuality of the sow, stage of lactation and feed have been shown to have an effect on the fat content of sow's milk. The animals were milked as completely as possible to try to prevent any variation because of first- and last-drawn milk. In this case, no breed difference was obtained between Poland China and Chester White milk. However, other factors mentioned by Reece, as well as methods of analysis, might cause a variation between results of different workers. The decrease in fat percentage of sow's milk as lactation advanced was the opposite trend to that shown by cow's milk. This may indicate that milk production remains at a high level

in sows for the 8 weeks lactation period. As lactation in sows is stopped suddenly by weaning the litter, it is not entirely comparable with lactation in cows.

The vitamin A level of sow's milk fat in changing from colostrum to milk followed the same general trend as in other species, namely, decreasing fairly rapidly. In sows the level of vitamin A in colostrum was only 4 to 5 times as high as in normal milk. This is much different from the colostrum of cattle, where the vitamin A content of normal milk is one twentieth or less of the initial concentration of vitamin A in colostrum, as reported by Stewart and McCallum (14) and Hansen *et al.* (8).

As lactation advanced, the spread between the vitamin A content of the milk fat in the pasture sows and dry-lot sows became wider. A depletion of the stored vitamin A in the dry-lot sows could account for the gradual decrease of vitamin A in their milk, whereas it tended to rise as lactation advanced in sows carried through gestation on pasture. There was no correlation between the number of pigs weaned or the weight of the litters weaned with the fat or vitamin A content of the milk within groups.

Shaw (12) has reported that injection of oxytocin in cows caused no significant differences in production of milk and milk fat over a 9-day period. Smith (13) found an increase in fat content for the first milking when oxytocin was injected. From this it may be assumed that the milk obtained from sows injected with oxytocin is approximately normal, although it might be slightly high in fat. The sampling error involved in milking by this method is much smaller than by attempting to get milk by hand without oxytocin, as complete evacuation of the mammary gland in sows cannot be obtained without the injection of oxytocin.

SUMMARY AND CONCLUSIONS

1. The fat and vitamin A levels of colostrum and milk are reported for the entire lactational period for ten sows carried through growth and gestation on pasture, and ten sows carried through growth and gestation in dry lot.

2. A gradual decline in fat content of the milk as lactation advanced was indicated in both groups.

3. An increase in vitamin A content of the milk fat toward the end of lactation was evident in the pasture sows, but a reverse trend occurred in the dry-lot sows. The mean values for fat were: first-day colostrum from pasture sows 6.73 per cent, from dry-lot sows 6.45 per cent; normal milk from pasture sows 6.12 per cent, from dry-lot sows 7.45 per cent of the milk. The mean values for vitamin A were: first-day colostrum from pasture sows 40.23 γ per g. of fat, from dry-lot sows 25.41 γ per g. of fat; normal milk from pasture sows 10.93 γ per g. fat, from dry-lot sows 5.69 γ per g. of fat.

4. No breed difference in fat content of the milk or vitamin A content of the fat was apparent between Chester White and Poland China sows.

REFERENCES

- (1) BENHAM, G. H. Vitamin A and New-born Pigs. Can. J. Comp. Med., 7 (10): 291-297. 1943.

- (2) BOWLAND, J. P., GRUMMER, R. H., PHILLIPS, P. H., AND BOHSTEDT, G. The Vitamin A and Vitamin C Content of Sow's Colostrum and Milk. *J. Animal Sci.* In press. 1948.
- (3) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. *Ind. Eng. Chem., Ann. Ed.*, 16: 101-102. 1944.
- (4) BRAUDE, R., COATES, M. E., HENRY, K. M., KON, S. K., ROWLAND, S. J., THOMPSON, S. Y., AND WALKER, D. M. A Study of the Composition of Sow's Milk. *Brit. J. Nutrition* 1 (1): 64-77. 1947.
- (5) BRAUDE, R., KON, S. K., AND THOMPSON, S. Y. A Note on Certain Vitamins of Sow's Colostrum. *J. Dairy Research*, 14 (3): 414-419. 1946.
- (6) DAVIES, W. L. The Chemistry of Milk. 534 Pp. Chapman and Hall, Ltd., London. 1939.
- (7) ELY, F., AND PETERSEN, W. E. Factors Involved in the Ejection of Milk. *J. Dairy Sci.*, 24: 211-223. 1941.
- (8) HANSEN, R. G., PHILLIPS, P. H., AND SMITH, V. R. Colostrum Milk and Its Vitamin A Content. *J. Dairy Sci.*, 29 (12): 809-814. 1946.
- (9) HENRY, W. A., AND WOLL, F. W. On the Yield and Composition of Sow's Milk. *Wis. Expt. Sta. Rept.*, p. 10. 1897.
- (10) HUGHES, E. H., AND HART, G. H. Production and Composition of Sow's Milk. *J. Nutrition*, 9: 311-322. 1935.
- (11) REECE, R. P. Factors that Affect the Fat Content of Milk. *N. J. State College Agr. Ext. Bull.* 238. 1943.
- (12) SCHOFIELD, F. W., INGLE, R. T., AND MCFARLANE, D. W. Vitamin A Assays of the Milk of Nursing Sows. *Rept. Ontario Vet. College for 1941*, Pp. 16-19. 1942.
- (13) SHAW, J. C. The Effect of Oxytocin on Milk and Milk Fat Secretion. *J. Dairy Sci.*, 25 (12): 1051-1055. 1942.
- (14) SMITH, V. R. The Effect of Milking at Short Intervals With and Without Injections of Oxytocin. *J. Dairy Sci.*, 30 (9): 703-715. 1947.
- (15) STEWART, J., AND MCCALLUM, J. W. "White Scour" in Calves and Related Infections. 1. The Significance of Vitamin A Content of the Colostrum as a Predisposing Factor in the Causation of Such Conditions. *J. Comp. Path.*, 51: 290-295. 1938.
- (16) WILLETT, E. L., AND MARUYAMA, C. The Effect of Intake of Garbage Fat upon Fat Content of Sow's Milk. *J. Animal Sci.*, 5: 365-370. 1946.
- (17) WOLL, F. W. The Composition of Sow's Milk. *Wis. Expt. Sta. Rept.* P. 267. 1899.

THE VIABILITY OF *BRUCELLA ABORTUS* IN MILK AND IN CREAM DURING HEAT TREATMENT IN ELECTRICALLY OPERATED HOME PASTEURIZERS¹

I. FOREST HUDDLESON, BETTY BALTZER, AND G. M. TROUT

*Departments of Bacteriology and Public Health and Dairy
Michigan Agricultural Experiment Station, East Lansing*

The presence of *Brucella abortus*, or the possibility of its presence in milk and cream coming from herds of dairy cattle which contain animals infected with the *Brucella* organism (Bang's disease), is an additional reason for the pasteurization of milk and cream destined for human consumption. The ingestion of *Brucella* infective dairy products is known to be one of the chief modes of transmitting brucellosis (undulant fever) to humans. While a large percentage of the milk and cream that is being distributed in the United States through commercial channels is pasteurized and, as a consequence, is free from living *Brucella* organisms, this cannot be said of a large amount of dairy products produced and used by farm families. In order to break one of the links in the infection chain of the *Brucella* organism where it is present in animals on a farm, it is essential that milk and cream consumed on such a farm be pasteurized.

The recent development of small, economical and easy-to-operate electric pasteurizers has brought to the farmer a means of not only treating milk and cream to preserve their keeping qualities, but to render these dairy products free from pathogenic bacteria.

Certain types of home electric pasteurizers have been studied by Trout and Bortree (2) to determine their ease and time of operation, the destruction of contaminating bacteria and the effect upon the milk itself. It was shown in this study that three types of pasteurizers, if operated according to the directions of the manufacturers, reduced the initial number of bacteria present in milk 82 to 99 per cent, depending upon the number and type initially present. The flavor of the milk or its creaming property was not impaired appreciably by the treatment.

From the data obtained by Trout and Bortree (2), there was no indication that *Brucella* organisms would not be killed if present in milk and cream during heat treatment in the electric pasteurizers. It seemed desirable, however, to have available specific information on this point, especially on cream of different butterfat concentrations, since most of the *Brucella* organisms are concentrated in cream during its separation from whole milk.

EXPERIMENTAL PROCEDURE

Two of the types of electric pasteurizers used by Trout and Bortree (2) were selected for this study, as they represented extreme differences in construction and operation. One is known as the Waters Conley pail type and the

Received for publication August 12, 1948.

¹ Journal article no. 976 -ns, Michigan Agricultural Experiment Station.

TABLE 1

Time and temperature required to kill all Br. abortus in whole milk, using pail type electric pasteurizer (3000 ml. sample)

Heating time	Recorded temperature	<i>Br. abortus</i> colony count
(min.)	(° C.)	(1000's/ml.)
0	19	127.0
10	31	122.5
15	39	128.2
20	46	101.5
25	54	121.2
30	61	12.2
40	68	0
50	68	0
70	69	0

All *Brucella* killed within 10 min. in effective temperature range.

other as the Wright in-the-bottle type. Their construction and method of operation have been described by Schaezner and Shiozawa (1).

In order to determine as closely as possible the efficacy of the two machines for killing *Br. abortus* during the time of operation, measured amounts of commercially pasteurized milk and cream were inoculated with a standardized suspension of a laboratory strain of *Br. abortus*. Raw milk and raw cream could not be used in the study, as they contained too large a number of contaminating bacteria, the presence of which made it difficult to make an accurate plate count of the inoculated *Brucella* organisms during the initial period of treatment.

The volume of milk or cream used for inoculation and heat treatment was 1 l. for the bottle type and 1.5 to 3 l. for the pail type of pasteurizer. Approximately 100,000 live organisms in suspension (plate colony count) were added for each milliliter of liquid. To insure thorough distribution of the organisms, the inoculated samples were stirred with a mechanical stirrer for 15 minutes before heat treatment. Samples of milk or cream were collected from the containers and diluted 1:100 in sterile diluting liquid in order to estimate the number of viable organisms present before heat treatment and the number remaining at 2, 5 or 10 minute intervals during the period of heat treatment.

TABLE 2

Time and temperature required to kill all Br. abortus in 20 per cent cream, using pail-type, electric pasteurizer (1500 ml. sample)

Heating time	Recorded temperature	<i>Br. abortus</i> colony count
(min.)	(° C.)	(1000's/ml.)
0	36	115.0
10	55	100.0
12	64	40.0
15	70	0
20	71	0
30	69	0
40	72	0
50	69	0

All *Brucella* killed within 6 min. in effective temperature range.

TABLE 3

Time and temperature required to kill all Br. abortus in 40 per cent cream, using pail-type electric pasteurizer (2000 ml. sample)

Heating time	Recorded temperature	<i>Br. abortus</i> colony count
(min.)	(° C.)	(1000's/ml.)
0	36	77.5
10	53	81.5
13	60	63.0
15	63	0
20	67	0
30	67	0
40	68	0
50	71	0

All *Brucella* killed within 5 min. in effective temperature range.

Plate counts were made on crystal violet tryptose agar using 1 ml. of the 1:100 dilution.

RESULTS

The electric timing control on both machines was set to begin operating when the temperature of the liquids reached 60–61° C. The electric heat control circuit on the pail type was broken automatically 40 minutes after a killing temperature was reached, and the one on the bottle type 30 minutes later.

In tables 1, 2 and 3 are recorded the results of the experiments obtained with the Waters Conley pail type pasteurizer. The length of time that was required for this pasteurizer to kill all the *Brucella* organisms added to milk or cream after reaching 60° C. varied from 5 to 10 minutes. The maximum temperatures recorded toward the end of the treatment period varied from 69 to 72° C. These experiments were performed three times with approximately the same results.

The results obtained with the Wright in-the-bottle pasteurizer are set forth in tables 4, 5 and 6. The length of time that was required for this type of pasteurizer to kill all added *Br. abortus* organisms after reaching an effective temperature (60° C.) varied from 5 to 8 minutes. The maximum temperature recorded toward the end of the treatment period was 64° C. Similar results were obtained in two additional experiments on inoculated milk and cream.

TABLE 4

Time and temperature required to kill all Br. abortus in whole milk, using bottle-type electric pasteurizer (1000 ml. sample)

Heating time	Recorded temperature	<i>Br. abortus</i> colony count
(min.)	(° C.)	(1000's/ml.)
0		119.5
30	45	100.7
52	60	35.5
54	59	27.5
56	61	2.8
58	62	0.1
60	62	0
64	62	0
84	64	0

All *Brucella* killed within 8 min. in effective temperature range.

TABLE 5

Time and temperature required to kill Br. abortus in 20 per cent cream, using bottle-type electric pasteurizer (1000 ml. sample)

Heating time	Recorded temperature	<i>Br. abortus</i> colony count
(min.)	(° C.)	(1000's/ml.)
0	25	122.5
15	34	94.0
20	38	94.0
25	41	97.5
30	45	90.0
35	49	87.5
40	53	77.2
45	56	79.5
50	60	7.7
55	62	0
60	63	0
80	64	0

All *Brucella* killed within 5 min. in effective temperature range.

TABLE 6

Time and temperature required to kill all Br. abortus in 40 per cent cream, using bottle-type electric pasteurizer (1000 ml. sample)

Heating time	Recorded temperature	<i>Br. abortus</i> colony count
(min.)	(° C.)	(1000's/ml.)
0	25	107.5
15	34	155.0
20	38	87.5
25	41	111.5
30	45	112.5
35	49	87.5
40	53	85.7
45	56	87.5
50	60	30.5
55	62	0
60	63	0
80	64	0

All *Brucella* killed within 5 min. in effective temperature range.

In view of the fact that heat killing temperatures were maintained for 25–30 minutes after live *Brucella* organisms could not be detected in 1 ml. amounts of either milk or cream, it would appear that a wide operating safety factor is provided by both types of pasteurizers.

SUMMARY

The data show that when milk or cream is inoculated with approximately 100,000 *Brucella abortus* organisms per ml. and treated in two different electrically operated milk pasteurizers for home use, according to the directions of the manufacturers, all *Brucella* organisms are killed within 5 to 10 minutes after the thermo-regulator begins to control the pre-set operating temperatures.

REFERENCES

- (1) SCHAEZNER, J. P., AND SHIOZAWA, S. Electric Milk Pasteurizers for Farm Dairies. Conv. Am. Soc. Agr. Engrs. Chicago. (Mimeo.) 11 pp. 1946.
- (2) TROUT, G. M., AND BORTREE, A. L. Laboratory Pasteurization Studies on Home Electric Milk Pasteurizers. Mich. Agr. Expt. Sta. Quart. Bull. 30 (1): 64–69. 1947.

DETERMINATION OF CAROTENE IN SILAGE¹

H. L. MITCHELL AND D. B. PARRISH

Kansas Agricultural Experiment Station, Manhattan

Since most of the carotene of the vegetative tissue of plants is found in the leaves, it is obvious that a heterogenous material such as silage will be difficult to sample for analytical purposes. In present methods, there are inadequate provisions for overcoming this difficulty. The method which has been used in this laboratory is a modification of the Peterson *et al.* method (2). The determinations usually were made in triplicate, with samples of 20 g. or more each, to reduce as much as possible the effect of non-homogeneity of silage. The modified method involved three extractions of the alkali-digested silage in a Waring blender, with Skellysolve B, alcohol and Skellysolve B, respectively. The extracts were combined and the alcohol was washed out with water. The Skellysolve B extract was concentrated to 30–40 ml., and the carotene was separated from other pigments by use of a magnesia adsorption column (3).

Mitchell and King (1) recently have proposed a method for determining carotene in alfalfa and cereal grasses which appreciably reduces sampling error. The method consists of drying the blanched or autoclaved plant tissue for 4 hours at 65° C., grinding the dried tissue and extracting the carotene from the resulting meal by the procedure of Silker *et al.* (3) for dehydrated alfalfa meal. Since a representative sample is obtained much more easily from the dried meal than from the chopped plant tissue, this method yielded reproducible results.

The method of Mitchell and King (1) has been compared with the modified procedure of Peterson *et al.* (2) to determine if it could be used for analysis of silage. Samples of sorghum silage were thoroughly mixed and quartered. One quarter was autoclaved for 10 minutes at 10 lb. pressure and dried in a circulating air oven for 4 hours at 65° C. Another quarter was dried without prior autoclaving. Three 20-g. samples were taken from a third quarter for carotene determination by the modified method of Peterson *et al.* (2). Samples were taken also for moisture determinations. The dried samples were ground to pass through a 20-mesh screen. Carotene was extracted from 5-g. samples of the meal by soaking overnight with 120 ml. of 30 per cent acetone in Skellysolve B. The remainder of the determination was conducted as specified by Silker *et al.* (3).

Comparisons of the results obtained by the two methods are presented in table 1. Replicates generally were in closer agreement by the Mitchell and King (1) procedure than by the modified Peterson *et al.* (2) method. In some instances, the variation by the latter method was such that an average of the three values for a given silage had little meaning. Grinding the undried silage with a food chopper or cutting it finely with scissors perhaps would have reduced sampling error, but this leads to uncertainty due to loss of tissue fluids.

Received for publication August 16, 1948.

¹ Contribution no. 370, Department of Chemistry.

As a further check on the method, carotene was determined by the modified method of Peterson *et al.* (2) on 5-g. samples of the meal obtained from the autoclaved portion of silage no. 6. The values obtained were 21.8, 23.5 and 24.3 $\mu\text{g.}$ (average 23.2 $\mu\text{g.}$) per g. dry weight. This compares satisfactorily with 21.7 $\mu\text{g.}$ obtained by the same method on the original silage and 23.6 $\mu\text{g.}$ obtained by the Mitchell and King (1) procedure on the meal (table 1). These values indicate that there could have been but little carotene destruction in the silage during drying.

The data of table 1 also show that autoclaving sorghum silage before drying for analysis did not result in appreciably higher carotene values than if the

TABLE 1

Comparison of the Mitchell and King procedure and the modified Peterson et al. method for the determination of carotene in sorghum silage

Sample	Modified Peterson, Hughes, Freeman		Mitchell and King			
			Unautoclaved		Autoclaved	
	($\mu\text{g./g.}^a$)	(av.)	($\mu\text{g./g.}^a$)	(av.)	($\mu\text{g./g.}^a$)	(av.)
1	45.3		53.7		53.4	
	46.7	46.0	55.8	54.7	54.4	53.9
2	30.2				32.5	
	33.6	31.9			32.5	32.5
3	43.5		43.1		45.9	
	54.3	51.1	45.9	44.5	47.6	46.8
	55.5					
4	54.3		42.7		46.7	
	58.8	57.9	42.7	42.7	48.5	47.6
	60.7					
5	58.2		44.7		47.7	
	77.7	76.7	45.4	45.0	48.6	48.2
	94.1					
6	19.9		19.9		23.3	
	22.2	21.7	20.1	20.0	24.0	23.6
	23.0					
7	15.6		14.3		14.0	
	17.5	17.5	14.7	14.5	14.8	14.4
	19.4					

^a Dry weight basis.

silage was dried without prior autoclaving. Sorghum silage differs in this respect from fresh alfalfa, for the latter must be blanched or autoclaved to prevent enzymic destruction of carotene during drying. Lack of carotene destruction during the drying of silage may be due either to absence of the enzyme in the original plant material, or to enzyme inactivation by the ensiling process. If the method is to be used with other types of silage, the material should be autoclaved before drying, unless it first is shown that such treatment does not result in higher carotene values.

Since it usually is necessary to make proximate analyses as well as carotene determinations on the silage, a further advantage of the procedure is that both can be made on the same dried sample. Such dried samples, if held at low

temperatures, need not be analyzed for carotene immediately. Hence, the method is adaptable to routine laboratory procedures.

SUMMARY

The Mitchell and King (1) procedure for determining carotene in fresh plant tissue is suitable for the determination of carotene in silage. Sampling error with this method is much less than with a modification of the Peterson *et al.* (2) method. Autoclaving sorghum silage before drying did not appear to be necessary in the analysis by this method.

REFERENCES

- (1) MITCHELL, H. I., AND KING, H. H. Determination of Carotene in Alfalfa and Cereal Grasses. *Anal. Chem.*, **20**: 637-638. 1948.
- (2) PETERSON, W. J., HUGHES, J. S., AND FREEMAN, H. F. Determination of Carotene in Forage. *Ind. Eng. Chem., Anal. Ed.*, **9**: 71-72. 1937.
- (3) SILKER, R. E., SCHRENK, W. G., AND KING, H. H. Determination of Carotene in Dehydrated Alfalfa Meal. A Simplified Method. *Ind. Eng. Chem., Anal. Ed.*, **16**: 513-515. 1944.

INFECTIOUS DISEASE AS A CAUSE OF INFERTILITY: A REVIEW¹

DAVID E. BARTLETT²

*Zoological Division, Bureau of Animal Industry,
Agricultural Research Center, Agricultural Research Administration,
U. S. Department of Agriculture, Beltsville, Md.*

INTRODUCTION

Diseases impairing reproduction strike at the fundamental operation of cattle husbandry. Heifers late in producing or never producing calves and cows producing calves at longer intervals than normal or never again producing calves, obviously fail to yield their entire potential profit. Fewer calves and lactations, prolonged lactations and dry periods, with maintenance costs constant or only moderately decreased, all contribute to the accumulated loss. Infectious diseases are productive of financial loss to the owner of affected cattle by interfering with coitus, by preventing conception temporarily or permanently, by interrupting pregnancy during any stage from fertilization to parturition, or by causing stillborn, moribund or feeble calves.

The ability to reproduce may be temporarily impaired during the course of any systemic condition associated with general debility or increase in body temperature; in bulls, spermatogenesis may be affected and quantity and quality of semen markedly altered, non-pregnant females may become anestrus, and pregnant females may abort. Diseases that are primarily systemic, however, are not within the scope of this paper. Discussion herein will be confined to those infectious diseases detrimental to reproduction that involve invasion by pathogenic organisms of some portion of the genitalia of either or both sexes. These diseases, in so far as their relationship to the genital system and its reproductive functions are concerned, are either (a) unspecialized or (b) specialized. The former are sporadic infections, either non-contagious or only slightly contagious, caused by organisms capable of causing disease of any part of animal bodies. The latter are contagious infections caused by organisms characterized by their specificity for particular species of animals and their decided predilection for, and more or less definite pattern of involvement of, the genital system.

Obviously, most genital infections do not result in absolute sterility. Some infections are of short duration and may leave no permanent effects. Other transient infections may leave lesions which impair or preclude reproduction. In bulls, malfunction of the testicles or accessory sex glands or occlusion of the tubular system may result. In females, extensive adhesions around the ovaries, occlusions of the oviducts, destruction of the functional lining of the uterus, and enlargement and distortion of the cervix all may occur. Some local infections may persist in the chronic stage and result directly in life-long, capricious fer-

¹ Based upon a paper presented in the symposium on Reproductive Problems of Dairy Cattle at the 43rd Annual Meeting.

² Now at School of Veterinary Medicine, University of Minnesota, University Farm, St. Paul 1, Minnesota.

tility. Still other infections, after a phase of activity characterized by inability to produce viable young for several years, become latent as a result of resistance acquired by the affected individual; thereafter, the ability to reproduce in an essentially normal fashion may be regained despite the existing infection. Some infections do not interfere with conception but prevent nidation, cause embryonal death, or interrupt pregnancies when well established. In such latter instances, the structures critically affected are those of the conceptus—the foetal placenta and the developing calf. Other infections, as a result of associated pain, may be responsible for diminished libido and consequent refusal of opportunity for coitus by bulls or resistance to coitus by females in estrum.

UNSPECIALIZED INFECTIONS OF THE REPRODUCTIVE SYSTEM

The unspecialized infections capable of causing reproductive failures usually require some predisposing condition in order to become established. They usually are local infections and frequently are of a type associated with production of pus. The organisms responsible occasionally may be transferred at coitus, but these are neither venereal diseases nor diseases that occur epizootically.

In females, lacerations such as those that sometimes occur at parturition or the conditions incident to decomposition of retained placentas may afford the opportunity for establishment of non-specific infections. In bulls, infections sometimes may ascend the urogenital tract. Also, in either sex, organisms may be brought to an area by the bloodstream and become localized. Vulvo-vaginitis cervicitis, metritis, salpingitis and ovarian bursitis in females and balanoposthitis, urethritis, seminal vesiculitis, inflammation of the other contributing genital glands, deferentitis and epididymo-orchitis in bulls may occur singly or in combination and in varying severity in the respective sexes. Streptococci, staphylococci, corynebacteria and *Pseudomonas pyocyaneus* are among the organisms which have been incriminated. Infection of the conceptus with termination of pregnancy, rarely in pyometra or more commonly in frank abortion, particularly in the later stages of gestation, may occur. Many unspecialized organisms, including streptococci, staphylococci, corynebacteria, diplococci, micrococci, molds and intestinal organisms, have been cultured from aborted fetuses (28).

The organisms concerned in these unspecialized diseases are ubiquitous in the environment of cattle and cannot be eradicated from a herd. Fortunately, these diseases are not among the most serious problems. They will continue to occur occasionally in herds despite the best management practices. Their treatment is largely symptomatic. That these diseases are diagnosed properly is very important in order that rational remedial measures may be employed and that applications of wrong therapeutic measures do not add further to the loss. Also, it is important that these diseases do not confuse the picture when infections that are specialized for the genital system are being dealt with.

SPECIALIZED INFECTIONS OF THE REPRODUCTIVE SYSTEM

Vesicular venereal disease (coital exanthema, genital pox) (14, 23). This is a highly transmissible, primarily venereal disease of cattle that occurs very rarely

in the United States, although it is reported rather commonly in Europe. Its etiological agent is believed to be a virus. This infection is characterized by its short incubation period of only 3 to 6 days and its lesions affecting the genital membranes. These begin as small papules and develop into lymph-containing vesicles that rupture and form ulcers which heal in about 2 weeks if uncomplicated. Affected individuals evidence considerable local pain; females may bawl and urinate frequently with much switching of their tails; bulls refuse coitus. Owing to its short course and prompt resolution, the effects upon fertility are not outstanding. However, individuals, particularly bulls, may be left with extensive adhesions requiring surgical intervention to restore coital function. The effects of this infection upon the uterus, germ cells and conceptus are unknown.

Treatment ordinarily is unnecessary, recovery being rapid and spontaneous. Individuals in which the inflammation is particularly acute may be benefited by irrigation with mild antiseptic solutions.

Although this disease is uncommon in its occurrence and not a cause of prolonged reproductive failure, it is important and merits restudy because of its potentialities. Vesicular venereal disease may become common in the United States at any time. Since it has been suggested that the virus of this disease is resistant outside the body, its relationship to artificial insemination should be thoroughly explored experimentally. Although this disease does not seem to have serious consequences, inadvertent transmission by artificial insemination would cause justifiable concern among cattle owners.

Bovine venereal trichomoniasis. Losses directly attributable to bovine venereal trichomoniasis, currently being experienced by cattle raisers of the United States, cannot be determined precisely, because the distribution and incidence of infected herds are unknown. Although this disease has been recognized in almost every State, only in Wisconsin have efforts been made to determine its occurrence and relative importance. Morgan, of the University of Wisconsin (19), reports that in the 6 years, from 1941 to 1946, 61 infected herds were found by the University's diagnostic service.

In the area within approximately 100 miles of the Agricultural Research Center, Beltsville, Maryland, the records of nine trichomonad-infected herds, having a total of more than 800 cattle, have been studied by members of the Zoological Division since 1934. Of these herds, four were purebred Aberdeen Angus; two were Guernsey, one purebred and one grade; and three were Holstein-Friesian, one purebred and two grade. These herds are in no way a significant indication of the occurrence of bovine venereal trichomoniasis in the Beltsville area, as no special effort has been made to locate infected herds. Their owners solicited the professional aid of either the University of Maryland Livestock Sanitary Service or the U. S. Bureau of Animal Industry. Nevertheless, the financial reverses experienced in these nine herds are believed to be highly significant. Two hundred thousand dollars is a conservative estimate of the loss of potential production of milk and calves and the loss of value of infected sires up to the time bovine venereal trichomoniasis was recognized as responsible for the reproductive failures in these infected herds (5).

This disease is caused by a protozoon, *Trichomonas foetus*, and is transmitted naturally only at coitus. However, mechanical transfer does occur readily between females through careless use of instruments or to females through artificial insemination with semen from infected bulls.

The direct deleterious effects of trichomoniasis are manifested almost entirely in the female, the uterus being the definitive site of infection. Infected females develop active immunity and ordinarily recover spontaneously. In bulls, *T. foetus* ordinarily lives on the surface of the preputial membranes and glans penis, produces no significant symptoms and ordinarily influences neither functional fertility nor potency. Bulls remain infected permanently, transferring trichomonads at practically every coitus.

In affected females, the most consistent effect is early termination of pregnancy, the relative time at which the developing conceptus is destroyed determining the symptoms displayed in individual cases. Most females, particularly those experiencing initial infections, return to estrum within 3 to 5 weeks *post coitum*. Trichomonad infection continues for several months during which time the females are infertile. Some females may abort recognizable fetuses during their first few months of pregnancy, in others the fetus may be liquified and pus may be accumulated in the uterus. This latter condition may persist for a year, superficially simulating pregnancy. On recovery from infection, most females are resistant to reinfection for a few years and may reproduce normally from coitus with infected bulls; eventually, however, the immunity is lost and reinfection occurs if coitus with trichomonad-infected bulls is permitted.

Diagnosis is based on actual demonstration of *T. foetus* in material collected from the genitalia of females or bulls.

The complete eradication of this disease from affected herds is the only effective means of handling this condition; ultimately, it is the most economical. Eradication involves the following steps: (a) Recognizing trichomonad-infected bulls and promptly withdrawing them from service (subsequent to successful treatment the previously infected valuable bulls may be restored to service) and (b) maintaining a constructive breeding program for all females in the herd, regardless of their status, systematically breeding each potentially infected female when she becomes free of infection and preventing her re-exposure incident to subsequent coitus.

Treatment of trichomonad-infected bulls is still on an experimental basis, but two methods subjected to limited but carefully controlled experimental trials conducted at the Zoological Division at Beltsville have shown considerable promise. These are (a) intravenous administration of large doses of sodium iodide (10 of 19 infections in 17 bulls were cured through experimental administration of 31 courses of iodides), and (b) topical application of a German-developed proprietary compound³ (ten infected bulls were treated and nine were cured by a single treatment; one remained infected despite two treatments). Details of progress to date with experimental treatments for trichomonad-infected bulls have been reported elsewhere (2, 3).

3 "Bovoflavin-Salbe"—Farbwerke Hoechst, Frankfort (m)-Hoechst.

Although results are encouraging, it is apparent that research has not yet progressed to a point where treatment of trichomonad-infected bulls can be recommended for general field use. Also, treatment of bulls is an exacting, lengthy, costly procedure requiring frequent handling and observation of the subject over a period of 6 months by a veterinarian familiar with trichomoniasis. Under practical field conditions, infected bulls not of exceptional value (at least three times their salvage value) as sires should be promptly slaughtered.

A procedure that has been found effective, experimentally, in systematically ridding seven of eight infected herds of bovine venereal trichomoniasis and the premise on which it is based have been fully described elsewhere by Bartlett and Dikmans (6). Application of this program necessitates extraordinary cooperation and understanding between the management of the infected herd and the attending veterinarian, for it is a laborious, long-term, expensive undertaking that requires much patience, many examinations, much careful bookkeeping and constant, alert herd management.

Herd owners introducing animals for breeding may afford their herds considerable protection by observing a few principles and precautions, namely: (a) Knowledge that the reproduction efficiency in the herd of origin is satisfactory is an excellent safeguard against selecting trichomonad-infected cattle. (b) Thorough examination of new suspect bulls by the diagnostic methods now available and examination of the first several females with which they are permitted coitus is indicated (4, 7, 8). (c) By prohibiting coitus of new, non-virgin, non-pregnant heifers and of new, non-pregnant cows that have had coitus since their latest parturition, introduction of trichomoniasis by females is precluded. The breeding of such females should be accomplished by artificial insemination only. New cows that have not had coitus since their latest normal parturition should be withheld from coitus until they have passed at least 2 estrums and rested at least 90 days postpartum.

Trichomoniasis may be disseminated readily to susceptible breeding females by artificial insemination, should semen from infected bulls be employed. The danger of unintentionally contributing to the spread of this disease merits constant serious consideration by responsible artificial insemination organizations. It is of extreme importance that the status of all bulls serving in such units be carefully determined by a competent diagnostician. It behooves herd owners to ascertain that the semen they are buying is from trichomonad-free bulls.

The outstanding need today for more effective control of bovine venereal trichomoniasis is a rapid, accurate, "one sample" means of determining the status of females and bulls, particularly the latter.

Bovine Vibrio fetus infection.—*Vibrio fetus* generally is recognized as a cause of occasional abortions in herds throughout the United States. Pregnancies may be terminated at almost any stage, but most frequently during the fourth to sixth month (second trimester) or seventh month. The manner of transmission is unknown. Precise knowledge of its incidence and epizootiology does not exist.

In the United States, abortion associated with vibrios was first described by Theobald Smith in 1918 (24). Later he reported that 26, or 23.8 per cent of a series of 109 abortions, originating from a large dairy herd in New Jersey, revealed *V. fetus* on bacteriological studies. Smith described the diseased state of the placentas of affected females. Subsequently, no significant contributions relative to cattle appeared until Plastring (20, 21) of Connecticut became interested in this condition. Recently, he reported studying 10 *V. fetus* infected herds in which the annual abortion rates attributed to *V. fetus* were 4 to 20 per cent and averaged 12 per cent. Also, Plastring observed a lowered conception rate in five of these herds. He has devised an experimental agglutination test for diagnosis.

Since the manner of transmission is unknown and the usual method of diagnosis difficult, rational means of coping with this disease in those herds in which it constitutes a problem are not available. Of course, general procedures in animal hygiene are indicated. All females in which abortion can be anticipated—and, ideally, all females at normal parturition—should be isolated and quarantined at least until genital discharges terminate.

It is evident that bovine *V. fetus* infection ordinarily is not responsible for the "storms" of abortion sometimes associated with bovine brucellosis. However, in the light of the present decline of bovine brucellosis, *V. fetus* as a cause of impaired reproduction today merits thorough experimental exploration.

Bovine brucellosis. Among the infectious diseases causing infertility, bovine brucellosis is still responsible for the greatest financial losses. Mingle (18), has estimated conservatively that in 1947 the beef and dairy cattle industries of the United States absorbed total losses of \$91,000,000 attributable to this disease. Bovine brucellosis and the problems incident to its control and eradication are well known; discussion in this brief paper would not be justified. Rational means of combating this disease are at hand. Their diligent application is indicated.

CONDITIONS OF QUESTIONABLE SPECIFICITY AND SPECIALIZATION

Nodular venereal disease (granular vaginitis, nodular vaginitis). To present an unprejudiced discussion of nodular venereal disease is exceedingly difficult. Pertinent to this subject one finds a paucity of established facts scattered among widely controversial opinions. This condition first received notice toward the close of the 19th century and was the subject of research by several workers. Since that time, much has been written regarding its importance and treatment, but no reports of comprehensive creditable research on its etiology and precise effects have been published. As far back as 1921, Williams (27) deplored the fact that this condition had not received serious study by pathologists and bacteriologists and pointed out that, "It may be said to be a lesion without a known cause and regarding the effect of which there is scant knowledge." That controversy still exists is evinced by recent literature where state-

ments were found that this disease is an outstanding cause of infertility (10, 13, 16), that although very common, only occasionally does this disease develop to the extent that it causes serious breeding troubles (9), and to the effect that this condition is of importance only to nostrum vendors in marketing their wares (23).

Some animals exhibiting the lesions ascribed to this condition can be found in most herds of cattle. In many females the lesions are confined to the vulva and may occur only about the clitoris, there being no involvement of the vagina. In other females the vulva and vagina may exhibit moderate to severe inflammation with rather general distribution of nodules. Lesions may be found in immature virgin heifers. It is not unusual to find severe lesions in females in all stages of pregnancy, whether their prior breeding records were normal or abnormal. On the preputial membrane and glans penis of bulls, nodules similar to those of females and inflammation may be found. Bacteriological studies have not revealed a specific organism consistently present in affected individuals. Neither has it been clearly demonstrated experimentally that this condition can be regularly reproduced artificially with consequent infertility nor has the contrary been demonstrated. It is possible that this condition is not primarily infectious.

Markedly affected individuals may be benefited by occasional douches with mild antiseptic solutions or, perhaps better, by occasional insufflation with absorbent antiseptic powders: Too vigorous or frequently repeated treatments are harmful. Application of treatments to an entire herd ordinarily is not justified.

There are admittedly many blanks in our present understanding of this condition. Nodular venereal disease should be the subject of a serious comprehensive research program designed to determine its cause, effect upon the reproductive capacities of the bovine and remedy.

MISCELLANEOUS INFECTIONS OF UNDETERMINED SPECIALIZATION AND UNKNOWN IMPORTANCE

Several species of molds have been reported recovered from aborted fetuses by a number of workers (15). Recently, from England, Rollinson and Haq (22) reported they found the same mold harbored in the prepuce of a bull that they found *post abortum* in the cervical mucus of a cow whose pregnancy had been initiated earlier by their coitus.

From England, pleuropneumonia-like organisms recently have been reported by Edward *et al.* (11) to have been isolated from the genital tracts of cows and bulls in herds without other ascribable cause for infertility. Affected females sometimes had cervicitis and adhesions of ovarian bursae.

From Holland a disease termed "enzootic sterility" has been described by Sjollem (23) and Ter Borg (25) and probably is the same as that described earlier by Webster (26) from New Zealand. This condition is described as a venereally-transmitted streptococcic affection of the uterus, cervix and vagina associated with return to estrum and early abortions.

In England several writers (1, 12, 17) have described a condition of breeding females known as "whites" associated with sterility and characterized by genital discharge post coitum and postpartum. Some claim this condition is caused by *Corynebacterium pyogenes* and is spread by coitus.

These latter fragmentary reports warrant further investigation. If the observations of the writers are confirmed, thorough experimental exploration is indicated.

GENERAL DISCUSSION AND RECOMMENDATIONS

For some infectious diseases, relatively effective prophylactic and combative procedures have been developed; dairymen and veterinarians need only to take cognizance of the established precepts. However, our present inability to cope rationally with certain other conditions and the meagerness of the knowledge of their total effects and causes must be recognized. Well directed, well supported scientific research is the only source from which the necessary facts can be obtained. Such research merits the encouragement, interest and support of the cattle industry and veterinary profession alike. It is obvious that the causes of lowered reproductive efficiency are exceedingly numerous and the problems of achieving optimum reproductive efficiency exceedingly complex and broad in their ramifications. No panaceas must be expected. Precise diagnoses whenever possible, rational corrective actions when indicated and trained, intellectually honest advisors mark the sound course. There should be no place for the triflers, "five-day specialists," self-confident, self-styled, handyman experts and quack remedy peddlers parasitizing and exploiting the field today.

For the cattleman, consistent observance of sound management and animal hygiene practices is imperative. Before introduction of breeding animals from outside herds, it is well to investigate their individual breeding records and also the general reproductive performance of their herds of origin. The keeping of precise, permanent records, together with a system of regular, physical examinations for pregnancy, will reveal any general occurrence of reproductive failures at the onset and by permitting early treatment or disposal of affected animals, will keep to a minimum the economic reverses resulting from infertility. Accurate, individual records are extremely valuable to the veterinarian in diagnosis and in planning treatment. Isolation of females when impending abortions are apparent and isolation of females after abortion and at calving time, at least until discharges cease, are indicated practices.

REFERENCES

- (1) ANONYMOUS. The control of Certain Diseases of Dairy Cattle, Vet. Record, 54: 194-196. 1942.
- (2) BARTLETT, D. E. Experimental Treatment of *Trichomonas foetus* Infection in Bulls, Am. J. Vet. Research, 7: 417-431. 1946.
- (3) BARTLETT, D. E. Further Observations on Experimental Treatment of *Trichomonas foetus* Infections in Bulls. Am. J. Vet. Research, 9: 351-359. 1948.
- (4) BARTLETT, D. E. Summary of Procedures for Diagnosing Bovine Venereal Trichomoniasis and Handling Affected Herds. J. Am. Vet. Med. Assoc. (in press).

- (5) BARTLETT, D. E. Unpublished data in files of Zoological Division.
- (6) BARTLETT, D. E., AND DIKMANS, G. Field Studies on Bovine Venereal Trichomoniasis: Effect on Herds and Efficacy of Certain Practices in Control. *Am. J. Vet. Research.* (in press).
- (7) BARTLETT, D. E., AND HAMMOND, D. M. Pattern of Fluctuations in Numbers of *Trichomonas foetus* Occurring in the Bovine Vagina During Initial Infections. II. Application in Diagnosis. *Am. J. Vet. Research*, 6: 91-95. 1945.
- (8) BARTLETT, D. E., HASSON, E. V., AND TEETER, K. G. Occurrence of *Trichomonas foetus* in Preputial Samples from Infected Bulls: Application in Diagnosis. *J. Am. Vet. Med. Assoc.*, 110: 114-120. 1947.
- (9) BOYD, W. L. Disease, Biggest Cause of Shy Breeding. *Successful Farming*, 45: 24. 1947.
- (10) CUNKELMAN, J. W. The Clinical Diagnosis and Treatment of Breeding Unsoundness in Cows. *J. Am. Vet. Med. Assoc.*, 112: 295-297. 1948.
- (11) EDWARD, D. G., HANCOCK, J. L., AND HIGNETT, S. L. Pleuropneumonia-like Organisms from the Bovine Genital Tract. *Vet. Record*, 59: 329-330. 1947.
- (12) HIGNETT, S. L. Bovine Sterility. *Vet. Record*, 52: 361-368. 1940.
- (13) HORNADAY, W. A. Sterility in Cattle. *Vet. Med.*, 42: 129-133. 1947.
- (14) HUTYRA, F., MAREK, J., AND MANNINGER, R. Special Pathology and Therapeutics of the Diseases of Domestic Animals. Vol. 1. Alexander Eger, Chicago. 1938.
- (15) JUNGHERER, E. Mycotic Affections of the Bovine Reproductive System. *J. Am. Vet. Med. Assoc.*, 86: 64-75. 1935.
- (16) KRILL, W. R. Granular Vaginitis and Sterility. *North Am. Vet.*, 29: 308-309. 1948.
- (17) McEWEN, A. D. Notes Concerning Abortion, Mastitis and Sterility. *Vet. Record*, 54: 155-157. 1942.
- (18) MINGLE, C. K. Bureau of Animal Industry. Personal communication. 1948.
- (19) MORGAN, B. B. Personal communication.
- (20) PLASTRIDGE, W. M., AND WILLIAMS, L. F. Observations on *Vibrio fetus* in Cattle. *J. Am. Vet. Med. Assoc.*, 102: 89-95. 1943.
- (21) PLASTRIDGE, W. M., WILLIAMS, L. F., AND PETRIE, DOROTHY. Vibrionic Abortion in Cattle. *Am. J. Vet. Research*, 8: 178-183. 1947.
- (22) ROLLINSON, D. H. L., AND HAQ, I. Mycotic Infection of the Prepuce of the Bull. *Vet. Record*, 60: 69-70. 1948.
- (23) SJOLLEMA, P. Onvruchtbaarheid Enzootische Steriliteit Bij Het Rund. *Tijdschr. Diergeneesk.*, 68: 508-515. 1941.
- (24) SMITH, T. Spirilla Associated with Disease of the Fetal Membranes in Cattle (Infectious Abortion). *J. Exptl. Med.*, 28: 701-719. 1918.
- (25) TER BORG, H. Enzootische Steriliteit Bij Het Rund. *Tijdschr. Diergeneesk.*, 70: 40-54. 1943.
- (26) WEBSTER, W. M. Bovine Sterility in New Zealand. *Australian Vet. J.*, 8: 199-222. 1932.
- (27) WILLIAMS, W. L. Diseases of the Genital Organs of Domestic Animals, 1st edn. The Author, Ithaca, N. Y. 1921.
- (28) WILLIAMS, W. L. Diseases of the Genital Organs of Domestic Animals. 2nd edn. The Author, Ithaca, N. Y. 1939.

HORMONES AND THE TREATMENT OF STERILITY IN DAIRY CATTLE: A REVIEW*

S. A. ASDELL

Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.

During the past few years considerable attention has been paid to the use of hormones as means of correcting infertility in live stock. In many animals in which disease is not very evident, derangements of the normal estrous cycle occur which should be amenable to this type of treatment. These include cases in which the cycle does not occur at all, possibly because the anterior pituitary gland is not functioning correctly or because the corpus luteum persists, preventing the regular cycle of events. Another type of hormonal disorder is found in the nymphomaniac in which the Graafian follicle fails to rupture and becomes cystic, thus causing the cycle to be interrupted. It also has been suggested that many cows with fairly regular cycles fail to conceive or to carry their calves to term because the corpus luteum or the uterus is not performing its function efficiently. Further, it has been suggested that resistance to diseases of the genital tract may be increased in some instances by reinforcing the normal hormonal mechanism. In this review, a list of the hormones available, their major functions and their best sources is given. This is followed by a statement of the point of view adopted by the writer and by an analysis of reports of the attempted use of these hormones in experimental work and in the field. This last part of the review deals with these problems as they are seen to occur in the field, *i.e.* the clinical condition is the basis of classification of the literature. As this part of the review is developed, certain gaps in our knowledge are pointed out as suggestions of the lines along which further data should be sought if progress is to be made. At the outset, it may be stated that the greatest need at present is for good assay methods so that hormone levels may be determined on individual cows. This is an important requisite if rational hormonal treatment is to become possible. Too much of the work, so far, has been along empirical lines, due not to shortcomings in the investigators but to their lack of effective tools.

HORMONES AVAILABLE

I. Anterior pituitary gonadotrophes

- a. Follicle stimulating hormone, causes growth of the Graafian follicle (after an antrum has been established) and spermatogenesis. Best source, horse pituitary; very little in cattle pituitaries, and not much in sheep or hog pituitaries.
- b. Luteinizing hormone, causes ovulation of a ripe Graafian follicle and formation of the corpus luteum, and also causes the cells of Leydig to secrete testosterone in the male. Best source, sheep and hog pituitaries.

* Based upon a paper presented in the symposium on Reproductive Problems of Dairy Cattle at the 43rd Annual Meeting.

c. Prolactin, causes the corpus luteum to secrete progesterone. No known function in the male.

These hormones are proteins and are not likely to be synthesized for a long time, so the only source is from pituitary glands. Extracts of the glands contain a mixture of these hormones. Prolactin is separated from the others fairly readily and can be purified with relative ease. The other two are not separated readily and pure preparations, though they have been made, are not available. The writer's experience has been that pure luteinizing hormone can be prepared but not in sufficient quantity for use. Follicle-stimulating hormone is so soluble that it is not readily obtained pure. McShan and Meyer (34) destroy the luteinizing hormone of extracts by tryptic digestion which leaves the follicle stimulator more or less unattacked.

II. Other gonadotrophins

a. Chorionic gonadotrophin from the urine of pregnant women. Entirely luteinizing in its action.

b. Human ovariectomy or menopause urine. Entirely follicle stimulating in its action.

c. Equine gonadotrophin from the blood serum of mares between 45 and 150 days of pregnancy. Entirely follicle stimulating in its action.

One of the main difficulties in the use of gonadotrophic hormones in animals with their pituitaries intact (a method of procedure which is obligatory in therapy) is that all these hormones are acting in the presence of the animal's own pituitary hormones which tend to modify the activity of the injected material. Also, by their action on the ovary or testis, the balance is altered for better or worse.

III. Sex steroids.

a. Estrogens. These hormones, secreted by the theca interna cells of the Graafian follicle and by the placenta, cause the psychic symptoms of heat, increase the blood supply to the endometrium, activate the myometrium and reduce the alkalinity of the vaginal secretion, thus promoting the liquifaction of the uterine seal. Estradiol is the most potent, then estrone and estriol. They are slightly soluble in water and much more so in oil, and usually are injected in the latter medium. This causes slow absorption and, in practice, as they are given as esters (e.g., estradiol monobenzoate) absorption is very slow, indeed. These hormones also cause the pituitary to change from follicle stimulating to luteinizing hormone secretion.

b. Progesterone, secreted by the corpus luteum, causes glandular growth of the endometrium and, in general, inhibits the activity of estrogens. It also is essential for the implantation of the embryo and probably is essential in the cow for the maintenance of pregnancy for the greater part of the period of gestation.

c. Androgens. These are secreted by the cells of Leydig of the testis. They are responsible for sexual desire and for the maintenance of the accessory

sex organs in the male. Testosterone is the most powerful, followed by androsterone.

All these natural sterioids are obtained from animal sources, estrogens from pregnancy urine, androgens from the urine of males and progesterone from corpora lutea. They also are synthesized, especially in the case of progesterone, beginning with certain sterols of vegetable origin. The use of natural estrogens rapidly is being replaced by the synthetic estrogens of group IV, below.

IV. Synthetic estrogens.

These do not occur in nature and are relatively simple in structure. They also have the advantage that they may be fed, not being destroyed in the gut as are the natural estrogens to a large extent. They have the biological properties of the natural estrogens.

a. Diethylstilbestrol (stilbestrol).

b. Hexestrol.

c. Octofollin.

d. Dienestrol, now being tried extensively, as it is more water soluble than the preceding and consequently is absorbed more easily, with a correspondingly more rapid and shorter action.

Our experience with diethyl stilbestrol has been that it is so slowly absorbed and excreted that it has a prolonged action, which may be detrimental to the return of normal sexual function. All the estrogens, both natural and synthetic, if given over a sufficiently long period cause nymphomania, with the usual symptoms, sinking round the tail head and liability to bone fracture, found in chronic cases. If given in pregnancy in sufficiently large doses, they cause abortion.

V. Other hormones

a. Oxytocin. This hormone, secreted by the posterior pituitary gland, causes the myometrium to contract. It is used either as such, or in the crude preparation, pituitrin, to hasten birth or to expel the retained placenta. Estrogens, by their action in stimulating the spontaneous activity of the myometrium, have much the same effect.

b. Thyroxin. This hormone, secreted by the thyroid gland, controls the basal metabolism and thus is essential for the efficient function of every cell of the body. There is evidence from gynecology that hypothyroidism often is associated with infertility, so this hormone must be considered in any account of hormone therapy. A synthetic form, thyroprotein, is available. It has the advantage over most hormones that it is not destroyed by the digestive juices, so it may be fed to the animal to be treated.

THERAPEUTIC USE OF HORMONES

The philosophy of the writer regarding the therapeutic use of hormones in the treatment of infertility stems largely from observations in an experiment made to test the usefulness of estrogens for this purpose (Asdell *et al.*, 7). In

this work, cows free from brucellosis, with no obvious signs of disease or abnormality and with fairly regular heat periods were treated with estrogens. The reason for selecting this method of treatment was that, since heat periods were fairly regular, the pituitary and ovaries probably were functioning normally but that the uterus was at fault. Estrogens were indicated since they increase the blood supply to the endometrium, thus giving a chance for better nutrition of that organ, and since they also stimulate the myometrium, thus improving the chance of normal function if that part of the uterus were at fault. The cows were bred naturally whenever they were in heat. Forty-two per cent of the treated cows conceived, but a control group had been included in the design of the experiment, and, of these, 50 per cent conceived in a fairly reasonable time. It is obvious that both in field trials and in experimental work this factor of spontaneous recovery must be taken into account. Adequate controlling is essential and it very rarely is found. The extent to which spontaneous recovery may affect the deductions varies with the criterion of sterility. Diagnosis of sterility by one veterinarian was followed by 12.5 per cent of spontaneous recovery; in our experiment, which may be regarded as fairly typical of the usual diagnosis in the type of infertility treated, the spontaneous recovery rate was 50 per cent; spontaneous recovery following diagnosis by another veterinarian was 85 per cent. This variation emphasizes the need for adequate controls in each trial which is made.

Further reflection pointed to the lack of logic in injecting hormones in empirical amounts when no information was available concerning the normal requirements of the cow and little on what various doses do to the cow's reproductive tract. Since that time, this situation has been corrected to a certain extent and the present knowledge on this subject has been brought together by Asdell (6).

Autopsy of the cows which did not conceive showed that, in most cases, disease of an erosive type had been present in the uterus, had damaged the cotyledons and then had cleared up. This also was borne out by the rather high abortion rate in the cows which conceived. Experience in this and in other instances leads the writer to believe that hormonal dysfunction is usually the result of disease factors which have upset the normal balance. In many cases, the structural damage which has been done is such that repair cannot be made. Primary hormonal imbalance in dairy cattle is by no means common. Functional sterility usually is the result of disease or of malnutrition.

In some types of infertility, the number of services needed for conception after treatment may be used with caution as a criterion of success, but even here a controlled experiment is preferable.

There has been too much tendency to apply hormones to all types of infertility on a shotgun basis without attempting to fit the treatment to the symptoms. As a result of this and due to the lack of controls, most of the work that has been done, both in the laboratory and in the field, must be taken with considerable reserve. In the account which follows, an attempt has been made to assess the reliability of the information given bearing this in mind. The method

of presentation is to consider different types of infertility separately. In practice, a combined therapy aimed at elimination of the casual agent and at rectification of the hormonal imbalance is indicated, but where this method has been used, assessment of the value of the hormonal therapy becomes problematical.

Generally speaking, the earlier literature is far more optimistic than the more recent. The earlier workers, particularly those on the European continent, often report "cures," apparently meaning that the symptoms have disappeared without indicating that the all-important conception has followed. Furthermore, in many reports, including recent ones, a few case histories are given describing successful treatments but with no reference to unsuccessful attempts.

THE FREEMARTIN

Attempts by the writer to modify the freemartin by the injection of estrogens have failed. In the four cases examined, the Mullerian ducts had lost the power of response to estrogens. As the gonad usually is a rudimentary cryptorchid testis, treatment with gonadotrophes is not indicated. Small gonads with ovarian stroma, but devoid of germinal epithelium, sometimes are observed but probably would not respond to gonadotrophes sufficiently to become functional.

INFANTILE OVARIES

Infantile ovaries, with the consequent failure of the accessory organs to develop, are due to a variety of causes. They may be due to late development, genetic in nature, or to failure for other reasons not yet understood, but usually the condition follows malnutrition in early life. The condition seems to be more frequent in more northern latitudes and lack of sunlight may be a factor. This may not be the primary factor involved, since high latitudes suffer from prolonged winters with consequent lack of succulent feed and a prolonged dry feeding period. The condition usually is found in heifers towards the end of winter, and it often is diagnosed when a number of heifers have been running with the bull and a check discloses very few conceptions. This means that treatment usually is initiated in early spring, when feed and climatic conditions are improving. Successes are credited to the treatment without sufficient basis.

The logical treatment is to inject a gonadotrophic preparation, preferably one with follicle stimulating properties, such as equine gonadotrophin or horse pituitary. The reaction of the normal heifer calf before puberty has been investigated by Casida *et al.* (15) who used pituitary extracts, both fractionated and unfractionated. They found that a calf whose ovarian follicles had not developed antra failed to respond. A similar result has been obtained by Smith *et al.* (50) in the rat. Further investigation may show that this is a serious limitation to the activity of gonadotrophes. Casida *et al.* (15) also found that there is a real danger of producing superovulations and that the eggs shed have a poor capacity for fertilization. Zawadowsky and Eskin (59) have found that conceptions are rare in cows inseminated at the first heat following the injection of chorionic gonadotrophin (prolan). However, if the reproductive system

can be stimulated sufficiently, spontaneous ovulations subsequent to the induced one should be normal.

Most of the earlier workers used gonadotrophins, but the later ones have been relying upon the synthetic estrogen, diethylstilbestrol. The mode of action, if any, of this hormone is obscure. Several workers suggest that it acts by "jolting" the anterior pituitary, causing it to initiate a normal rhythm in the reproductive tract. Comment on this is reserved until the experimental results have been given.

Pighini (43) treated one heifer by grafting anterior pituitary tissue and injecting a crude anterior pituitary extract. She conceived. Asdell (5) treated 2 heifers with a crude anterior pituitary extract from sheep and both conceived, but not until they had been on grass for some time. Eisenbach (21) treated 15 heifers with 125 R.U. of chorionic gonadotrophin and 87 per cent conceived. Amicare (3) treated 1 heifer with 325 R.U. of chorionic gonadotrophin without effect. Bottomley *et al.* (11) treated one heifer with chorionic gonadotrophins without result. Teunissen (55) treated 42 heifers with chorionic gonadotrophin; 26 came in heat and 18 conceived. He also treated 16 with equine gonadotrophin; 9 came in heat and 5 conceived. This gives 78 heifers treated with gonadotrophes with a pregnancy rate of 55 per cent.

Steinach *et al.* (53) treated 19 heifers with estradiol benzoate (Progyon B), 50,000 M.U. in one injection. Of these, 95 per cent came in heat within 2-4 days and 53 per cent conceived. Anderson and Bugg (4) injected 15 mg. of diethylstilbestrol dipropionate (the usual form in which this hormone is administered) into six heifers with small inactive ovaries. One came in heat, but none became pregnant. Allen (21) injected 18 heifers with the same substance and seven became pregnant. He notes that if heat was observed after the injections the chances of success were good and that the less the bodily development the poorer the outlook. Wright (56) injected 23 heifers with from 1,500 to 3,000 I.U. of stilbestrol; 5 came in heat and none conceived. It is not clear how long Wright persisted in his attempt to obtain pregnancy. Entirely negative results in experiments or trials of this nature are as difficult to explain as is 100 per cent success. If his report is omitted, 43 cows have been treated with estrogens with 40 per cent success.

On the basis of published results estrogen treatment of cows with infantile ovaries has been less successful than gonadotrophin treatment. Casida *et al.* (15), in their work with normal heifers, found the most successful treatment to be an initial injection of a follicle stimulator followed by an injection of luteinizer. This is a rational treatment, but it has not been followed in the field so far as published results show. Indeed, most workers have relied entirely upon luteinizer.

The idea that stilbestrol jolts the anterior pituitary into action is not supported by the results of this form of therapy. The effect of this substance upon the pituitary of the immature animal has not been worked out. More information is needed along these lines and also more is needed upon the condition of

the ovaries of those heifers which show no response, if there be one, to any form of therapy.

HYPOPLASTIC OVARIES IN THE ADULT

The condition of ovarian hypoplasia in the adult has points of similarity to that of infantile ovaries, but the condition found seems to be more variable. One cause is malnutrition. The ovaries may vary from a condition in which small follicles are present, without heat periods and ovulations, to a complete fibrous degeneration. The rational hormonal treatment is to inject a follicle stimulator, and, in some cases, this is reinforced by the classical massage treatment. Reports on treatment are numerous. Perhaps it might be as well to begin their consideration with a report by Clark (16) that of 15 treated by massage alone, 14 conceived.

Spieler (51) treated ten cows with a mixed estrogen and anterior pituitary preparation (hormovilan) and nine conceived. Asdell *et al.* (7) treated three cows with a sheep anterior pituitary extract and two conceived. Hupka and Majert (27) gave 60 cows one or two injections of 125-200 R.U. of prolactin (chorionic gonadotrophin), a very small dose by more recent standards, and 62 per cent came in heat, while 47 per cent conceived. They also report the injection of 20 cows, with 90 per cent in heat after one to five injections. Bettini (10) treated nine cows of which eight were "cured." Amicare (3) treated eight cows with 125 R.U. of prolactin and all conceived. Eisenbach (21) treated six cows similarly and all were "cured." Menzani (35) treated 25 cows and 96 per cent came in heat. Pataki (42) treated 19 cows and 84 per cent came in heat. Koch (31) treated 34 cows which had anaphrodisia, a sequel to hoof and mouth disease. They received 250 R.U. of prolactin, and conception at the first mating was recorded in 19 of them. Bottomley *et al.* (11) treated 19 cows and 6 conceived. Haisch (25) treated 12 and 11 conceived. Teunissen (55) treated 32 cows and 10 conceived. This gives a record of 165 cows treated with chorionic gonadotrophin with 50 per cent conceptions.

Kedrov (29) reports heat after treating cows with atrophic ovaries caused by underfeeding. His treatment consisted of injecting 1,000 M.U. of equine gonadotrophin, and he remarks that few of them conceived. Teunissen (55) treated two cows with the same type of preparation and both conceived.

Treatments with estrogens also have been numerous. Murphey *et al.* (38) treated two cows with an estrogen extract and both conceived. Steinach *et al.* (53) treated 66 cows by injecting 50,000 R.U. of estradiol benzoate (Progynon B) and 95 per cent came in heat. Bennewitz (9) gave 18 cows the same treatment; all came in heat and 54 per cent conceived. Ratti (46) reported heat in 44 of 55 cows similarly treated. Küpper (32) reported 36 treated with 20 in heat and 14 pregnant with another estrogen (Unden). Allen (2) treated four cows and obtained four pregnancies, while Mirskaja and Kedrov (36) treated one group of seven cows with 16 to 30 mg. of stilbestrol and reported six in heat and one pregnancy. They treated 16 cows which did not come in heat after parturition in a similar manner. Four of these ovulated but only one

conceived. Teunissen (55) treated four with another estrogen (Dimenformon) without success. Finally, Zollinger (6) treated 36 cows with stilbestrol and 58.8 per cent conceived. The total number of cows treated with estrogens is 123 and, of these, 53 per cent conceived.

Amongst miscellaneous types of hormone treatment of this type of infertility may be mentioned a report by Deubler and Barnes (19), who treated 19 cows by injecting an extract of one or two ovaries or by feeding extracts of two ovaries in a capsule. Eighteen of these cows came in heat, but, as natural estrogens are not absorbed from the gut in small doses, the successes cannot have been due to estrogens. Stäheli (52) treated 51 cows by transplanting one or two ovaries. Of these cows 85 per cent came in heat and 61 per cent conceived. Frei and Stäheli (23) treated nine cows with "vethormone" of which six came in heat and five conceived. This substance is a mixture of ovarian, pituitary, thyroid and pancreatic hormones.

SUBESTRUM

The English workers have described a condition which they term "subestrum." Animals which are classified thus do not come in heat, and small follicles are present in the ovaries but no corpora lutea. It appears to be a mild form of ovarian hypoplasia, and the type of treatment suggested should be the same as for that disorder.

Pataki (42) treated nine cows with 62 to 125 R.U. of chorionic gonadotrophin and seven conceived. Jensen (28) combined this treatment with uterine douches and ovarian massage. Of 47 cases treated, 46 came in heat and 79 per cent conceived. Zavadovskii (58) treated 92 cows with equine gonadotrophin. Heat was recorded in 56 and pregnancy in 39. Wright (57) treated 18 cows with 15 to 25 mg. of stilbestrol. Seven came in heat regularly after the treatment but none conceived. Durrell (20) treated twelve with semen in the cervix and three conceived. A cow treated with equine gonadotrophin conceived, but with testosterone propionate only one of six became pregnant. One cow treated with progesterone conceived.

PERSISTENT CORPORA LUTEA

The usual treatment for persistent corpora lutea is to remove these bodies by squeezing them from the ovaries. In some cases, attempts have been made to treat with hormones. Spieler (51) used an anterior pituitary extract in 20 cases with 14 conceptions. Hupka and Majert (27) treated ten with small doses of chorionic gonadotrophin and seven came in heat. Menzani (35) used the same treatment in 20 cases, and 13 cows came in heat. Eisenbach (21) reported all cured with 21 treatments, while Teunissen (55) obtained three pregnancies in six treatments with the same substance. Cameron (12) treated 46 cows with equine gonadotrophin. Twenty-one came in heat, and he obtained some pregnancies.

Estrogens also have been used in this condition. Bennewitz (9) treated 15 cows with 50,000 R.U. of estradiol benzoate (progyon B); 94 per cent came

in heat, 75 per cent conceived, and 20 per cent became nymphomaniac. Küpper (32) treated 13 cows with a similar dose (Unden) and obtained seven pregnancies, mostly after one to two sterile matings. Mirskaya and Kedrov (36) treated 13 cows with estrogens but no pregnancies resulted.

ABSENCE OF HEAT, CAUSE NOT GIVEN

These cases of anaphrodisia may be due to ovarian hypoplasia or to persistent corpora lutea. Hancock (26) treated six cows with chorionic gonadotrophin and three conceived. Murray (39) used equine gonadotrophin (1,500 I.U.) in 19 cases, and 9 of these showed ovarian reaction. He also treated 35 cows with stilbestrol (20 mg.). Heat followed in a large proportion, but only one conceived. Glenney (24) also treated 12 with stilbestrol and two pregnancies resulted. Lentz (33) treated six with estrogens (folliculin) and four conceived.

NYMPHOMANIA

Nymphomania usually is treated by removal of the follicular cysts which cause the condition. In cases of long standing the cysts frequently have lost their lining of granulosa cells, and this presents a problem in hormonal treatment, since the rational hormonal therapy is to luteinize the follicles, thus breaking the deadlock in the cycle. Another complication may be the involvement of the central nervous system in the syndrome, as nymphomania is said to occur occasionally in the absence of large amounts of estrogens (Alba and Asdell, 1). An important paper on the condition and its treatment with hormones is that by Casida *et al.* (14). They point out that when extracts of sheep pituitary are injected the results differ with the site of injection. Subcutaneous injections produce follicular growth, while intravenous injections cause luteinization. Thus, the latter method is the one to be recommended in this particular form of therapy.

A wide variety of hormonal treatments have been attempted. Asdell (5) injected an extract of sheep pituitaries in four cases and obtained one conception. Walsh (56) injected twelve cows and seven conceived, while in two others normal cycles were restored. Casida *et al.* (14) made a very thorough test of this method using intravenous injections of sheep pituitary extract. They divided their cows into two groups of nymphomaniacs. Group 1 consisted of 71 cows which were nymphomaniac but without uterine complications. In 55 of them, corpora lutea formed, 52 showed normal heats and 46 were bred, with 32 pregnancies. Group 2 consisted of ten cows in which there were uterine complications. In this group, corpora lutea were formed in nine; normal heats followed in seven but none became pregnant. The only criticism which can be made is that many of the cows were in the incipient stages of nymphomania and the spontaneous recovery rate was not known. These workers also report that in 33 of the cows of group 1, cysts were ruptured, but that the degree of recovery in these cows did not differ from those in which the cysts were not ruptured. The total for this treatment is 97 cows injected, with 41 per cent of conceptions.

A luteinizing hormone, chorionic gonadotrophin, has been used by many workers. Koch (30) treated 35 cases and reported success in 30, with pregnancies in several. Niklas (41) treated 31 cows with 100 to 400 R.U. and had 20 cures, 11 of which were only temporary. He also reports treatment of 40, with 65 per cent conceptions. Haisch (25) treated nine cows and obtained six conceptions; Hancock (26) treated five with three conceptions; Deubler (18) had five injected cows and all conceived. Moore (37) treated 18; 12 ceased to be nymphomaniac, 10 had corpora lutea and 12 conceived. Teunissen (55) treated 12 cows and none conceived, while Durrell (20) records six treated, six conceptions. Lentz (33) used Folluetin and reported 16 conceptions in 17 cows injected. The total for this form of treatment is 112 cows of which 66 per cent conceived.

Teunissen (55) has used equine gonadotrophin in three cases, but none conceived.

Estrogen treatments also have been tried. Murphey *et al.* (38) treated one cow without success. Lentz (33) used stilbestrol in one case without success. Dancey (17) used the same substance on four cows; three improved and two conceived.

Progesterone has been used by Carlson (13), who recorded five pregnancies in five cases. Smith (49) treated ten cows and all symptoms disappeared. Bellomo (8) records the "effective" treatment of five cases with progesterone.

Durrell (20) has tried testosterone propionate in two cases without success.

One gains the impression from some of these reports that other forms of treatment than hormonal injections alone have been resorted to and this makes several of the most optimistic reports difficult to accept at their face value.

CYSTIC OVARIES WITHOUT NYMPHOMANIA

The exact significance of this classification is somewhat obscure. It may include cases in which the cysts occur in the corpora lutea or in the ovarian adnexa (mesovarium, parovarium). Spieler (51) treated 22 cows with an anterior pituitary extract and 77 per cent conceived. Casida *et al.* (14) used a similar treatment intravenously in 13 cases; nine produced corpora lutea, ten had normal heats and four conceived. Deubler (18) treated two cows with chorionic gonadotrophin and both conceived.

COWS WITH HEAT PERIODS BUT NO CONCEPTION

The type of cow in which heat periods are regular to irregular presents a most important problem, because they give the opportunity for breeding, but much time is wasted due to the delays in getting them pregnant. From the investigators point of view, they pose difficult problems, since, in the absence of obvious lesions, it is difficult to know when to begin treatment as sterile cases. Probably many of the cows with irregular heats represent a group of early aborters.

A further difficulty in assessing the results of treatment lies in the fact that in these cows their state of relative infertility may represent the degree of fer-

tility of the bull to some extent. A relatively infertile bull is not so successful with this class of cow as is a very fertile one. A rational hormonal method of treatment is not obvious, and many kinds of therapy have been attempted. In this class of infertility, controls are more essential, if that is possible, than in any other, and they rarely are found.

Spieler (51) treated 15 cows with an anterior pituitary extract and 93 per cent conceived. Asdell *et al.* (7) treated 11 cows with a sheep pituitary extract and four conceived. These cows had failed with previous estrogen treatment.

Eisenbach (21) used chorionic gonadotrophin in the small dose of 125 R.U. He reported that 12 cows were treated and all were cured. Bottomley *et al.* (11) treated 19 cows with 2,500 R.U. and obtained six pregnancies. In a group of cows in which difficulty was experienced in obtaining conceptions, the percentage of successful services was compared in treated and untreated cows. For the experimental cows services with treatment were 57 per cent effective, for untreated services 7 per cent, while for control cows, not deemed in need of treatment, services were 58 per cent successful. The authors draw the conclusion that treatment restored the average fertility to the level of the controls. Durrell (20) treated ten cows and four conceived.

Durrell (20) also has treated 24 cows with equine gonadotrophin, and 11 conceived to the first heat during or following therapy, a result which he interprets as showing the beneficial effects of the injections. He also treated three cows with equine gonadotrophin and progesterone and obtained one conception.

The value of estrogens has been studied by several workers. Murphey *et al.* (38) treated six cows with estrogens and four conceived. Frank (22) used ovarian extract (without corpora lutea) on 35 heifers and 90 per cent conceived. In 50 cows, about 90 per cent conceived to the first service and 10 per cent to the second. Zupp and Murphey (61) treated two cows without success. Spieler (51) used a complex mixture containing estrogens and obtained 93 per cent conceptions in 15 cows. Risse (47) used the same preparation (hormovilan) on 85 cows and reported 16 per cent conceptions. Asdell (5) used estradiol benzoate in eight cases and two conceived. Clark (16) treated 41 cows which had averaged 4.6 unsuccessful matings with a saline douche, and 38 conceived after an average of 1.9 further matings. He also used saline douches and injected an ovarian extract in 17 cases which had previously been treated with saline douches. Ten conceptions resulted in this group. Mirskaya and Kedrov (36) treated 11 cows with estrogens and only one pregnancy followed. Asdell *et al.* (7) injected 31 cows with estradiol benzoate and ten conceived. This is 32 per cent. Of 18 controls, 55 per cent conceived. The first group, as it is reported here, contained several cows which had failed to conceive as controls and which were treated subsequently. The average number of services per conception in the treated cows that became pregnant was 3.0, while in the control group (without treatment), 1.7 services were required. The evidence from this experiment neither supports the view that estrogens improve fertility nor that it lowers the number of services required per conception. In one field

test in which the cows treated were regarded as poor prospects, two of ten conceived. In another herd 17 cows were treated, 15 of them conceived but five aborted subsequently. Accuracy of diagnosis thus is an important factor in assessing results, especially in this type of infertility.

Durrell (20) treated four cows with progesterone and obtained one conception. Ruegg (48) fed testes to eight cows, seven became normal and four conceived. It is improbable that any hormone was absorbed by these cows.

At this stage, it hardly seems necessary to comment further upon this group of infertile cows.

OTHER CONDITIONS IN THE COW LEADING TO INFERTILITY

Metritis is a causal factor, or a contributory one, in many cases of infertility. Haisch (25) treated 25 cows with Lugol's solution together with injections of 25 R.U. of chorionic gonadotrophin. He states that 19 were cured. Spieler (51) treated seven cows with an anterior pituitary extract and six conceived. Anderson and Bugg (4) treated three with stilbestrol. One was improved and two not. Glenney (24) reports that stilbestrol has some effect in improving cows with pyometra.

Haisch (25) treated 23 cows suffering from vaginal prolapse with 250-1,000 R.U. of chorionic gonadotrophin and reports 17 cured and four others improved.

Stilbestrol has been recommended as an aid to the expulsion of the retained mummified fetus. Stuart (54) treated one cow with 25 mg., together with posterior pituitary extract. The fetus was expelled. One cow treated with stilbestrol alone failed to respond. Anderson and Bugg (4) also failed with one cow. Powell (45) tried one cow with chorionic gonadotrophin without effect; later she responded to stilbestrol. Murray and Robertson (40) treated one cow with stilbestrol without effect.

SEXUAL INACTIVITY IN BULLS

A good deal of work has been done on the use of various hormones upon sterility and sexual inactivity in bulls, but very little of it has been reported in the literature. In general, it may be said that, judging from results obtained with other species, when spermatogenesis ceases entirely the prognosis is unfavorable.

Pighini (43) treated eight bulls with anterior pituitary grafts and six became active again. Bottomley *et al.* (11) treated three bulls which produced semen with low sperm motility by injecting chorionic gonadotrophin and all were improved. Durrell (20) treated four bulls with low ability to mount with 300 mg. of testosterone propionate; all responded favorably, but in one the response was temporary. Reineke (46) treated 14 bulls with thyroprotein. Ten were improved in their libido, and conception rate improved in four of them.

GENERAL REMARKS

It has been a depressing task to bring the literature on the hormonal treatment of sterility together. Very little of the work is controlled adequately, and

much of it is anecdotal in character. The complications in work of this nature are such that it is difficult to devise critical experiments, but in view of the importance of sterility much more of this critical type of work should be done. Where adequate controls have been set up, hormonal treatment has failed to demonstrate efficacy. Another feature is that in any definite type of infertility the average percentage of response is about the same whatever the treatment. Such uniformity seems to show that the type of treatment with these boundaries means little; other factors leading to a fairly uniform rate of recovery are at work. These conclusions do not mean that hormonal treatment necessarily is worthless. They mean that we know too little about the exact rôle played by the hormones and about the dosages that should be employed. Much more groundwork needs to be done and many more critical field trials. Also, in field trials, the worker has tended to neglect the recommendations of the physiologist. He has worked on a shotgun basis without fitting the treatment, in most cases, to the type of infertility encountered.

REFERENCES

- (1) ALBA, J. DE, AND ASDELL, S. A. Estrous Behavior and Hormones in the Cow. *J. Comp. Psychol.*, 39: 119-123. 1946.
- (2) ALLEN, G. S. Some Observations on Stilbestrol Dipropionate in the Treatment of Anoestrus. *Vet. Record*, 55: 168-169. 1943.
- (3) AMILCARE, P. Sulla Cura della Sterilità Bovine. *Profilassi*, 7: 347-350. 1934. (*Animal Breeding Abs.*, 3: 361. 1935.)
- (4) ANDERSON, G., AND BUGG, R. R. Observations on the Use of Stilbestrol Dipropionate. *Vet. Record*, 54: 249. 1942.
- (5) ASDELL, S. A. Some Practical Applications of Endocrinology to Animal Husbandry. *Am. Soc. Animal Production Proc.*, 28: 235-237. 1936.
- (6) ASDELL, S. A. Patterns of Mammalian Reproduction. Ithaca, N. Y., 1946.
- (7) ASDELL, S. A., FINCHER, M. G., SMITH, S. E., AND ELLIOTT, F. I. A Controlled Experiment to Restore Fertility in Dairy Cattle by Treatment with Gonadic and Gonadotropic Hormones. *Cornell Univ. Ag. Expt. Sta. Mem.* 243. 1942.
- (8) BELLOMO, G. Sull' Ormone del Corpo Luteo e sua Applicazione nella Terapia della Bovine Nimfomani. *Riv. Milit. Med. Vet.*, 5: 83-88. 1942. (*Animal Breeding Abs.*, 12: 76. 1944.)
- (9) BENNEWITZ, G. Behandlung Ovariell bedingter Unfruchtbarkeit bei Kühen, Farsen und Stuten mit dem Follikel-hormonpräparat Progynon B. *Diss.*, Hannover, 1936. (*Animal Breeding Abs.*, 7: 201. 1939.)
- (10) BETTINI, U. Sull' Impiego del Prolan nella Cura della Sterilità nella Vacca e nella Cavalla Sostenuta da Lesioni Ovariche. *Nuova Vet.*, 11: 500-503. 1933. (*Animal Breeding Abs.*, 2: 89. 1934.)
- (11) BOTTOMLEY, A. C., FOLLEY, S. J., AND WATSON, H. M. S. Experiments on the Use of Chorionic Gonadotrophin (Pregnancy Urine Extract) for the Treatment of Sterility in Dairy Cattle. *J. Agr. Sci.*, 30: 235-243. 1940.
- (12) CAMERON, H. S. Clinical Observations on the the Use of Equine Gonadotropin in the Mare and Cow. *J. Am. Vet. Med. Assoc.*, 100: 60-62. 1942.
- (13) CARLSON, E. R. Functional Sterility in Cattle. *Cornell Vet.*, 30: 427-436. 1940.
- (14) CASIDA, L. E., MCSHAN, W. H., AND MEYER, R. K. Effects of an Unfractionated Pituitary Extract upon Cystic Ovaries and Nymphomania in Cows. *J. Animal Sci.*, 3: 274-282. 1944.
- (15) CASIDA, L. E., MEYER, R. K., MCSHAN, W. H., AND WISNICKY, W. Effects of Pituitary

- Gonadotropins on the Ovaries and the Induction of Superfecundity in Cattle. *Am. J. Vet. Research*, 4: 76-94. 1943.
- (16) CLARK, C. F. Clinical Results in the Treatment of So-called Functional Sterility of Cows. *J. Am. Vet. Med. Assoc.*, 90: 488-492. 1937.
 - (17) DANCEY, J. C. Practical Experiences with Dibestranol D. P. *Can. J. Comp. Med. and Vet. Sci.*, 9: 23-25. 1937.
 - (18) DEUBLER, E. C. Antuitrin S in the Treatment of Cystic Ovaries of Cows and Mares. *Vet. Ext. Quart., Univ. Penn.*, 89: 31-39. 1943.
 - (19) DEUBLER, E. C., AND BARNES, M. F. Sterility in Cattle. *J. Am. Vet. Med. Assoc.*, 61: 254-258. 1922.
 - (20) DURRELL, W. B. Observations on Sterility in Dairy Cattle and the Use of Natural and Synthetic Hormones. *Can. J. Comp. Med. and Vet. Sci.*, 11: 290-297. 1947.
 - (21) EISENACH, F. A. Zwei Jahre 'Prolan' im Kampfe gegen die Sterilität der Rinder. *Wien. tierärztl. Monatsschr.*, 22: 202-204. 1935.
 - (22) FRANK, E. R. The Role of Ovarian Extract in Bovine Sterility. *Cornell Vet.*, 24: 75-79. 1934.
 - (23) FREI, W., AND STÄHELI, A. Über Anaphrodisie bei Kühen. *Deut. tierärztl. Wochschr.*, 34: 755-760. 1926.
 - (24) GLENNEY, W. C. Stilbestrol in Dairy Practice. *North Am. Veterinarian*, 25: 91-96. 1944.
 - (25) HAISCH, A. Versuche mit Prolan in Oel bei Konstitutionell bedingter Sterilität des Rindes, insbesondere bei Unterentwicklung der Ovarien, Nymphomanie, Metritis und Scheiderworf. *Berlin u. Münch. tierärztl. Wochschr.*, 46-47. 1944.
 - (26) HANCOCK, J. L. Clinical Observations on the Use of 'Luteinizing' Hormone (Chorionic Gonadotrophin) in the Treatment of Ovarian Cysts in the Bovine. *Vet. Record*, 59: 639-641. 1947.
 - (27) HUPKA, E., AND MAJERT, W. Ueber die Einwirkung des Prolan auf Eierstock und Euter bei Haustieren und über seine Eignung zur Bekämpfung der Sterilität dieser Tiere. *Deut. tierärztl. Wochschr.*, 40: 614-616. 1932.
 - (28) JENSEN, G. M. Ufruglbarketsbehandling hos Koeget specielt med Henblik paa Hormonpreparaters Avendelighet. *Medeensk. Danske. Dyrl.*, 15: 4. 1941. (*Animal Breeding Abs.*, 14: 20-21 1946.)
 - (29) KEDROV, V. Causes of Infertility in Cows and Measures for its Elimination. *Dokl. Akad. Selzkhoz. Nauk. Lenin.*, 1-2: 34-39. 1945. (*Animal Breeding Abs.*, 14: 83. 1946.)
 - (30) KOCH, W. Die Hormonbehandlung der Nymphomanie des Rindes. *Tierärztl. Rundschau*, 43: 451-454. 1937.
 - (31) KOCH, W. Hormonbehandlung der Ovaryhypoplasie nach Maul- und Klauenseuche. *Vorläufige Mitteilungen. Berlin u. München tierärztl. Wochschr.*, 576-577. 1938.
 - (32) KÜPPER, P. Über die Wirkung des Follikel-hormonpräparates 'Unden' auf den Weiblichen Geschlechtsapparat des Rindes bei Anaphrodisie. *Diss., Hannover*, 1938. (*Animal Breeding Abs.*, 8: 128. 1940.)
 - (33) LENTZ, W. J. Hormonal Therapy in Cystic Ovaries of Cows. *Vet. Ext. Quart., Univ. Penn.*, 95: 8-17. 1944.
 - (34) MCSHAN, W. H., AND MEYER, R. K. The Preparation and Properties of Pituitary Follicle-stimulating Fractions made by Trypsin Digestion. *J. Biol. Chem.*, 135: 473-482. 1940.
 - (35) MENZANI, C. Sull' Impiego degli Ormoni Sessuali Preipofisari ('Prolan') nel Trattamento dell' Ipofunzione Ovarica nella Bovine. *Nuova Vet.*, 12: 240-243. 1934. (*Animal Breeding Abs.*, 3: 21-22. 1935.)
 - (36) MIRSKAJA, L. M., AND KEDROV, V. K. Can Estrogens Restore Fertility in Cows? *Vestn. Zivotn.*, 1: 74-84. 1945. (*Animal Breeding Abs.*, 15: 174. 1947.)
 - (37) MOORE, G. R. Effects of Chorionic Gonadotropin on Cystic Ovaries in the Cow. *Vet. Med.*, 41: 163-164. 1946.

- (38) MURPHEY, H. S., McNUTT, G. W., ZUPP, B. A., AND AITKEN, W. A. Case Reports on the Use of Oestral Hormone and Other Gland Extracts in the Treatment of Functional Sterility. 7th Rept. J. Am. Vet. Med. Assoc., 68: 443-450. 1926.
- (39) MURRAY, J. G. Notes on the Subject of Infertility in Cattle. Vet. Record, 55: 323-326. 1943.
- (40) MURRAY, J. G., AND ROBERTSON, R. R. Treatment for Expulsion of Mummified Foetus. Vet. Record, 54: 484. 1942.
- (41) NIKLAS, F. A. E. Beitrag zur Anwendung des Prolan in öligler Aufschwemmung. Berlin. tierärztl. Wochschr., 54: 283. 1938.
- (42) PATAKI, P. Organotherapie im Kampfe gegen die Sterilität. Münch. tierärztl. Wochschr., 86: 15-17. 1935.
- (43) PIGHINI, G. Iniezioni ed Innesti di Ipofisi Anteriore in Animali Iposessuali e Senili. Nuova Vet., 11: 446-458. 1933. (Animal Breeding Abs., 2: 90. 1934.)
- (44) POWELL, C. R. A. Successful Ejection of a Mummified Foetus. Vet. Record, 54: 137. 1942.
- (45) RATTI, P. Die Provetanbehandlung der Sterilität beim Braunvieh (Bergell). Schweiz. Arch. Tierheilk., 79: 324-326. 1937.
- (46) REINEKE, E. P. The Effect of Synthetic Thyroprotein on Sterility in Bulls. In, The Problem of Fertility, E. T. Engle, Ed. Princeton. 1946.
- (47) RISSE, W. Behandlung der Unfruchtbarkeit mit Standardisiertem Weizenkeimöl (Vitamin E) und Hormovilan bei Rindern. Diss., Hannover. 1936. (Animal Breeding Abs., 7: 220-221. 1939.)
- (48) RUEGG, J. Über Versuche, den Antagonismus der Weiblichen und Männlichen Keimdrüsen Therapeutisch zu Verwenden. Schweiz. Arch. Tierheilk., 76: 2-12. 1934.
- (49) SMITH, C. L. Nymphomania in Dairy Cattle Treated with Corpus Luteum Extract. Vet. Med., 36: 530. 1941.
- (50) SMITH, P. E., ENGLE, E. T., AND TYNDALE, H. H. Differential Ovarian Responses after Injections of Follicle-stimulating and Pregnancy Urine in Very Young Female Rats. Proc. Soc. Exptl. Biol. Med., 31: 744-746. 1934.
- (51) SPIELER, H. Behandlung der Unfruchtbarkeit bei Rindern mit Hormovilan. Tierärztl. Rundschau, 41: 326-327. 1935.
- (52) STÄHELI, A. Über Eierstocksimplantationen bei Praesenilen Kuehen. Festschr. zum siebzigsten Geburtstage von Herrn Prof. Dr. E. Zschokke in Zürich, 85-92. 1925.
- (53) STEINACH, E., STÄHELI, A., AND GRÜTER, F. Behebung der Sterilität bei Landwirtschaftlichen Nutztieren (Rinder, Kühe, Schweine) durch das Weibliche Sexualhormon. Wien. klin. Wochschr., 47: 129-132. 1934.
- (54) STUART, K. Two Cases of Mummified Foetus Treated with Stilbestrol. Vet. Record, 54: 106. 1942.
- (55) TEUNISSEN, G. H. B. Anaphrodisie bij het Rund in het Bijzonder Betreffende de Resultaten van de Behandeling met Gestyl. Tijdschr. Diergeneesk., 71: 510-517. 1946.
- (56) WALSH, F. E. Dysfunction of the Ovary. J. Am. Vet. Med. Assoc., 101: 404-410. 1942.
- (57) WRIGHT, L. G. Observations on the Clinical Aspect of Reproductive Disorders in Cattle. Vet. Record, 57: 313-320. 1945.
- (58) ZAVADOVSKII, B. Utilization of P.M.S. for the Control of Infertility in Cows. Sovkoz. Proizvod., 6: 29-33. 1945. (Animal Breeding Abs., 14: 84. 1946.)
- (59) ZAWADOWSKY, M., AND ESKIN, I. New Data on the Regulation of the Sexual Cycle in the Cow. Bull. Biol. Med. Exptl. U.S.S.R., 4: 200-202. 1937. (Animal Breeding Abs., 7: 122. 1939.)
- (60) ZOLLINGER, M. Über Behandlung der Sterilität des Rindes mit Provetan-Schering und Stilbestrol-Roche. Diss., Zürich, 1946. (Animal Breeding Abs., 15: 176. 1947.)
- (61) ZUPP, B. A., AND MURPHEY, H. S. Study of the Phenomena of Oestrus and Reproduction in Domestic Animals with Case Reports. (8th Dept.) Vet. Med., 21: 261-264. 1926.

NUTRITION AND THE TREATMENT OF STERILITY IN DAIRY CATTLE: A REVIEW*

S. A. ASDELL

Laboratory of Animal Nutrition, Cornell University, Ithaca, New York

The relation between nutrition and infertility in dairy cattle is a difficult one to review because it has so many ramifications. The direct literature is rather scanty compared with that upon the same subject in laboratory animals; much may be gathered by inference but little can be substantiated by reference to critical work. Malnutrition may affect the reproductive system in a variety of ways, directly, and indirectly, and it may take a wide variety of forms. Under practical conditions, one rarely finds a clear case of malnutrition due to a deficiency of one factor in the feed. The usual condition found is that in which a multiple deficiency exists. Underfeeding may be accompanied by poor quality of the feed; thus, an energy deficiency often is complicated by deficiencies in protein, phosphorus and vitamin A. A protein deficiency often is accompanied by a phosphorus deficiency, and a vitamin A deficiency by a protein and phosphorus deficiency. When a specific deficiency leading to reproductive disorder is found, treatment consists of a review of the feeding picture and the application of common sense methods in providing better feed.

Malnutrition usually results in lowered vitality so that it is reasonable to assume that any form of malnutrition lessens the resistance to disease. This is one of those general statements that is very difficult to prove on an experimental basis. Attempts to prove it for the reproductive tract have been very few and they have led to negative or inconclusive results. When disease has once made inroads in an animal or herd, the problem is no longer the simple one of correcting feed conditions; the disease factors must be attacked and eliminated, as they are not only troublesome in themselves, but the affected animals are reservoirs which threaten the health of the other members of the herd.

In discussing the problems of reproduction with farmers, the writer always has stressed the fact that a healthy herd is more profitable, both in production and reproduction, than an unhealthy one, and that it is better to maintain a herd in good health by adequate feeding than it is to attempt to restore a herd that has been let down by malnutrition. When this stage has been reached, the problem is no longer the simple one of providing adequate feed. The fact that adequate feed is cheaper than attempts to restore deficiencies by reinforcing an inadequate dietary regime with proprietary articles also has been stressed.

In general, it has been shown for many deficiencies that the young animal is more susceptible than is the adult. Early malnutrition prevents the orderly growth of the reproductive organs as a physiologically integrated system. When this occurs, it is difficult, and sometimes impossible, with our present knowledge, to initiate the correct balance for proper development. Once reproduction has

* Based upon a paper presented in the symposium on Reproductive Problems of Dairy Cattle at the 43rd Annual Meeting.

begun the problem is easier, as it then is a question of maintaining in smooth operation an organization which already has been built up. The mature animal is more resistant, also, because it has reserves to draw upon which are not available to the growing animal. For these reasons, the writer believes that more attention should be paid to the nutrition of the growing animal, both male and female. Prevention rather than cure should be the keynote of our advisory and research work.

Specific dietary deficiencies rarely cause specific lesions in the reproductive tract. They usually produce general effects leading to a variety of types of infertility or of disease. Therefore, in this review the approach is to consider the food factors individually and to point out the types of failures that have been observed, together with the conditions under which deficiencies may occur. It is not feasible to classify by considering the individual functions of the reproductive organs. No attempt is made to review the relations between deficiency diseases and reproduction as a whole. Attention is centered upon the knowledge we possess of the subject as it relates to dairy cattle.

During the past few years, many attempts have been made, particularly in the management of bulls used for artificial insemination, to reinforce, in a variety of ways, rations usually considered adequate. These reports have been, in the main, negative, but Smirnov-Ugrjumov and Laptev (48) found that the addition of animal protein improved semen quality. However, Branton *et al.* (7) failed to find any benefit on semen quality or fertility when they used skim milk powder as the principal protein in a 15 per cent supplement. On the other hand, they also found that a poor protein, corn gluten feed, was without deleterious effect.

In interpreting results, the time factor is important, especially in bulls. It takes about 3 weeks for complete spermatogenesis and about 3 weeks more for transit through the epididymis, while sperm retain their fertilizing power when stored in the epididymis for a maximum of about 40 days. In view of these facts, it is unlikely that a deficient diet which causes a sudden break in spermatogenesis will have its full effect upon semen ejaculates and on fertility for several weeks after the break occurs. Aristotle (2) was led to deny that the testis had a direct bearing on fertility, because he had noticed that a certain bull did not lose his fertility immediately after castration.

GENERAL INANITION

It generally is believed that when calves are underfed they are late in attaining puberty, but there is little precise data on the subject. In fact, the average age of puberty for heifers under normal conditions never has been worked out with statistical detail, so that no "yardstick" exists by which comparisons might be made. There is a certain amount of anecdotal lore concerning the effects of underfeeding on the age of puberty, but, while most of this has a basis in fact, we probably are dealing with deficiencies of a multiple nature rather than with a straight energy deficiency. Richter (43) pointed out that heifers

were late coming in heat after the underfeeding which occurred during World War I, and Moehl (33) has reported in much the same vein. Eckles (12) records that heifers fed heavily experienced their first heat earlier than did those fed lightly; the difference for Jerseys was 65 days, for Ayrshires, 100 days, and for Holsteins, 126 days. That underfed heifers are late experiencing their first estrus also has been recorded by Allen (1) in England. The condition often shows itself towards the end of an unusually severe winter and is corrected when the heifers are placed on spring pasture. Quinlan (41) has reported similarly from South Africa. In older animals, under-nutrition results in irregular heat periods and low fertility (Richter, 43; Dolder, 11).

In bulls, Jones *et al.* (24) reported that calves fed after 5 to 7 months of age on alfalfa hay and minerals grew at a rate which was 10 to 15 per cent below normal. They did not produce good semen of normal fertility until they were 2 to 3.5 years old, while others fed likewise, but with the addition of skim milk powder, performed efficiently after they were 1 year old. They ascribed the difference in the two groups to a difference in energy intake.

There appears to be no data from dairy cattle dealing with specific deficiencies of protein, carbohydrate or fat.

MINERAL DEFICIENCIES

The usual deficiency met with in the field is a phosphorus deficiency. It tends to occur when diets low in protein are fed, under conditions more or less resembling those found on the range when the grass is dry or when the soil definitely is deficient in phosphorus, and in borderline areas, when the cows are lactating but are not fed an adequate protein and mineral supplement. Generally, reproduction does not appear to suffer until the animal shows clinical symptoms of phosphorus deficiency, *e.g.* unthriftiness, rough coat and depraved appetite. Tuff (53) reported reduced fertility in dairy cattle in certain areas of Norway in one of the earlier thorough studies devoted to this problem. Eckles *et al.* (13) studied conditions in affected areas in Minnesota. They reported that there was considerable breeding trouble; on many farms not more than one calf was obtained every 2 years, but there was not more than the usual amount of abortion. Heifers sometimes did not come in heat until they were past 2 years old. The condition was most severe in late winter and early spring, especially following a dry summer and fall. One cow which was killed had sub-normal ovaries, and palpation showed this condition to be general. Phosphate supplements cured the condition; cows ovulated and the normal number of conceptions occurred. Analyses showed that phosphorus was low in the feeds grown on affected farms, but the calcium content was normal. In a further study, Eckles *et al.* (14) stated that cows which were affected tended to have one or two heat periods after they calved. If they became pregnant at this time, the calves were carried to term; if not, they tended to show anestrus for the rest of their lactation; after they were dried off, heat periods returned and they had a normal chance of conception. In this connection, Derivaux (10) found that neither phosphorus nor calcium deficiencies affected the corpus luteum. Palmer

et al. (38), in an experimental study, found delayed first heats in heifers. Later, ovulations without heats occurred at normal intervals. The life of the corpus luteum was normal. Conception occurred with relative ease but parturition tended to be difficult and four of the eleven calves were either weak or dead at birth. This was an extreme case of phosphorus and protein deficiency, and the cows were about 50 per cent underweight.

Riddell *et al.* (44) also report absence of heats in cows on a phosphorus-deficient ration.

In South Africa, Quinlan *et al.* (42) found that the average age at first heat in 28 heifers fed dry rations and restricted in exercise was 528 days. When cycles were established, their length was normal. The deficiency here may be due to vitamin A lack and not to phosphorus. Theiler *et al.* (50) previously had reported a calf crop of 51 per cent in phosphorus-deficient herds, and this was restored to 80 per cent in herds with a phosphate supplement.

Webster (57) reported that in phosphorus deficient (Waihi disease) areas of New Zealand the calf crop was 63.7 per cent in pika herds and 68.2 per cent in no pika herds; this was not a very large difference, but supplements were being fed in many of the pika herds. The chief reproductive symptom was absence of heat periods, and three cases at autopsy had subnormal ovaries. He found a correlation of + 0.45 between the phosphorus content of spring pasture and the breeding efficiency index. The correlation between the latter and calcium content was -0.35, and for the Ca:P ratio it was -0.51. The correlations with autumn pasture were much lower. Phosphorus was not really low on any of the farms studied. Farms above the mean in Ca:P ratio had a breeding efficiency index of 1.6, while those below the mean had an index of 1.3.

It appears, therefore, that phosphorus deficiency interferes with ovarian function, causing probably a lowered estrogen secretion in the early stages. In more severe conditions, follicular development is interfered with. If pregnancy occurs, little effect is seen until the end of gestation, when parturition may be difficult and the calves may be born weak or dead. The deficiency occurs where phosphorus in the soil and rations is low, and it may only become apparent in borderline cases when the drain of phosphorus through lactation is rather heavy. Work is needed on the anterior pituitary to see whether the effect is through this organ or directly upon the ovary.

The phosphorus requirement for reproduction is about 10-12 g. per day. This is not above the requirement for growth but decidedly below the additional amount needed if the cow is lactating (Huffman *et al.*, 23).

In connection with the reports that once pregnancy is initiated it is not interrupted, it is interesting to note that Hart *et al.* (18) found that contagious abortion was no more frequent in a herd receiving poor rations without minerals than in one in which the rations were good and were reinforced with mineral supplements.

There is no evidence that a calcium deficiency causes reproductive failure in dairy cattle (Palmer *et al.*, 37; Huffman, 22). Trace elements also have not been incriminated as yet.

Iodine deficiency apparently does not impair the reproductive function in itself, so far as is known for dairy cattle in practice. It does, however, cause the birth of premature, weak or dead calves. According to Kalkus (25), the danger is greater in a prolonged winter and if the calves are born in a cold spell. Keith (26) has reported in much the same vein. The danger may arise in the Sierra region and in that of the Great Lakes.

VITAMINS

Vitamin A deficiency has been reported frequently in range cattle when they have been on dry feed or dried up range for some time. One such report is that of Hart and Guilbert (19). They include among the symptoms the birth of dead or weak calves with frequent retention of the placenta. The condition somewhat resembles that which is associated with brucellosis. The cows which produce these calves usually exhibit typical symptoms of vitamin A deficiency to some degree. Such symptoms are night blindness, ophthalmia and diarrhea. Lack of condition also is frequently noticed. These workers ascribe similar reproductive failures observed by others on diets consisting entirely of certain plants, such as wheat, oats or corn, to a lack of this vitamin. They also consider that similar failures, observed when cottonseed meal is fed, are due to the destruction of vitamin A. Meigs and Converse (31) have observed these symptoms in dairy cows fed dry rations. There is no evidence that estrous cycles are interfered with in either beef or dairy cattle. Thus, Davis and Madsen (9) obtained conceptions in beef cattle receiving less than 60 μ g. of carotene per kg. body weight. This amount was insufficient to prevent the development of typical lesions. They performed a post-mortem examination on one heifer that had aborted at 8 months and found that she had "infantile" ovaries and a cystic pituitary. The condition of the corpus luteum apparently has not been investigated.

Conception may not occur as readily in vitamin A deficient cows, as Axelsson (4) reports that cows kept for 3 months before service on a diet containing 27-47 mg. of carotene required 2.0 matings per conception; those fed 48-72 mg. required 1.4, and those above 72 mg. required 1.2 matings.

Vitamin A deficiency in bulls has received considerable attention. Sutton *et al.* (49) kept calves on a low vitamin A diet until they were a year old. They observed degeneration of germinal epithelium and absence of spermatozoa. The alpha cell zone in the anterior pituitary was extended, but, nevertheless, there was an increase in gonadotrophic potency of the tissue. Fluid had accumulated in the cleft of the pituitary. Hodgson *et al.* (21) kept 12 bulls on a diet which provided a low intake of this vitamin. Seven remained fertile though they would not mount in many cases. The semen was low in spermatozoan concentration, the count of abnormal sperms was high, the pH was higher than usual and the semen did not store well. They remarked that gross symptoms of deficiency appeared before reproductive function began to suffer. If the deficiency was produced before the onset of puberty, the bulls failed to breed at all. Their pituitaries were cystic and the epithelium of the seminiferous tubules was de-

generating. The addition of vitamin A to the diet did little to improve the reproductive organs once these lesions had appeared. Erb *et al.* (15) also found that vitamin A deficiency delayed puberty, decreased sex drive and reduced the amount of spermatogenesis. The anterior pituitary was edematous, and there were histological changes in the adrenal cortex. It was found that the sexual disorders continued long after the addition of adequate amounts of vitamin A to the diet. In both these papers, it was stated that gross symptoms preceded involvement of the reproductive organs. A film prepared by Bratton at Cornell shows that ability to mount cows is lost before semen production and fertility suffer to any marked extent.

Remarkable results have been claimed for the use of vitamin C (ascorbic acid) in the treatment of failures in reproduction, but so far the method has not been submitted to a critical test. In view of the fact that the greatest degree of success has been claimed in cases with regular cycles but without obvious pathology, and that it is in this class that spontaneous recoveries are most frequent, the need for adequate controls is obvious, but one does not find them. The writer's experiences with hormones, both in his own work and through the literature causes him to reserve judgment until critical data are available for study.

Phillips *et al.* (39) found that normally there is a rise in blood plasma ascorbic acid during mid-to-late heat. Of seven poor breeders, two failed to show this rise. Eleven cows with regular cycles or with a tendency to omit heats were injected with this vitamin. Ten of them conceived to the first service. In three cows which lacked uterine tone and in three with cystic ovaries, the cows failed to respond to the injections. These workers quote a communication from Brown who injected twelve cows and obtained seven or eight conceptions. McIntosh (30) advises the injection of 1.5 to 2 g. of ascorbic acid per 1000 lb. live weight. He cites a communication which he had received in which, of 33 cows with regular heats which had been injected, 31 conceived. Of six with irregular heats, four conceived, while no response was obtained in eight cows with cystic ovaries. Barker (5) records the case of one cow which conceived in 2 consecutive years on the day on which the vitamin was injected and three in which it was ineffective. It may be mentioned that average diestrous plasma ascorbic acid levels in 82 cows have been obtained by Asdell *et al.* (3). These cows required from one to six services per conception or failed to conceive after six services. No relationship was found between the ascorbic acid level and the ease of conception.

Injections of ascorbic acid are said to be equally effective in the treatment of infertility of the bull. Phillips *et al.* (40) treated 29 bulls with poor breeding records by injecting 1.5 g. of ascorbic acid per 1000 lb. live weight each week. Four failed to respond, and one of these had atrophic testes. The semen changed in consistency from a thin watery fluid to thick and creamy. The spermatozoa were increased in their longevity. Six of these bulls had little sex interest in cows and all were improved after the injections. These workers also found that in cases in which the ascorbic acid in the semen was below 2 mg. per 100 ml. the

bulls were poor breeders, from 2 to 8 mg. their record was satisfactory, while for those in which the level was above 8 mg. the breeding records were erratic.

One method of increasing the ascorbic acid level in the blood is by feeding chloretone. Scheidenhelm *et al.* (46) have used this method with three bulls which had poor records. In each of them the number of services needed to obtain conceptions was improved very markedly.

It is possible that vitamin D deficiency is a factor in bovine infertility. Wallis (56) reports a decline in general health when this substance is lacking in sufficient quantity and records that four cows on such a diet did not experience heat periods.

The problem of vitamin E need for adequate reproduction in cattle is a difficult one to review because so many extravagant claims have been made for the use of this compound. Usually these reports have dealt with the addition of the vitamin in the form of wheat germ oil or sprouted oats.

In 1931 Vogt-Muller and Bay (55) reported that they treated 12 cows, which did not conceive readily, by injecting 10 cc. of wheat germ oil intramuscularly. All conceived after the treatment. Later, Bay and Vogt-Muller (6) reported the treatment of 70 cows with 49 conceptions. Risse (45) treated 60 cows but only 10 conceived in 4 to 6 weeks after treatment. It had no effect at all on young and old cows with ovarian atrophy, persistent corpora lutea and follicular cysts. Koenen (27) reported some favorable results, but not enough to indicate that wheat germ oil is a specific remedy for infertility. Tutt (54) used wheat germ oil in 25 cases of infertility due to various conditions and obtained 17 conceptions. However, all these cases were undergoing other treatments at the time of injection, mainly in the form of douches with Lugol's solution. In this connection, and also in connection with ascorbic acid therapy, one must note that Clark (8) treated 41 cows which had averaged 4.6 unsuccessful matings with a saline douche, and that 38 of them conceived after an average of 1.9 further matings. Schweizer (47) fed vitamin E to 19 cows that failed to conceive for no apparent reason and 13 conceived. McIntosh (29) reports that of 57 cows treated with wheat germ oil 38 conceived at their first subsequent service. No details are given. Moussu (35) states that wheat germ oil prevents losses due to contagious abortion. It was given in 30-40 cc. doses at conception, at 3 and 6 months. In a district where 7,000 cows were thus treated, "to-day the success is regular and everywhere one sees abortions, non-deliveries and mortality of the new-born disappear".

On the other side of the picture is the report by Gullickson *et al.* (16) that nine heifers were raised on vitamin E-free rations and that all calved normally.

On the use of wheat germ oil for bulls, there are the reports by Timin and Pereturina (51, 52) of 5 bulls which were receiving steppe hay and concentrates. These rations were reinforced with 500 to 1200 g. of wheat embryos. Sexual activity improved, semen volume increased 18 per cent, sperm numbers increased 11 per cent and activity increased 14 per cent. Gullickson *et al.* (16) reported that two bulls fed a vitamin E-free ration were fertile. In five others raised on similar rations, the sexual development was normal.

Harris *et al.* (17) have used a vitamin E concentrate of mixed tocopherols. They report that in one herd a number of cows which had averaged five services per conception in previous years had a service rate of less than three in a year during which either the tocopheral supplement, or one containing this supplement together with vitamin A, was fed. The record for the previous year showed three stillbirths, four abortions, and retained placentas requiring the services of a veterinarian after the birth of almost every calf. In the year of supplementation, no stillbirths, one abortion and one retained placenta were recorded.

Moore (34) used sprouted oats on four heifers that did not conceive readily. Two conceived during the experimental feeding period. Miller and Graves (32) used 115 heifers and cows. Of these, 27 conceived before they were fed sprouted oats, 57 conceived during or after the treatment and 31 did not. Henke (20), in a thorough experiment with controls, did not find that sprouted oats were of benefit. He had one group of four heifers which had not come in heat at all. Two were fed sprouted oats and both conceived, though one did not until a year after the feeding had been stopped. Two were not fed sprouted oats and one conceived. Another group consisted of 19 cows that were slow in coming in heat after calving. Of nine fed the oats, all conceived. Of ten not fed in this manner, nine conceived. Each group averaged 2.2 services per conception, but the fed group averaged 268 days from calving to conception and the control group 229 days. Another group consisted of cows bred once without conception. Twenty-seven were fed sprouted oats, and 20 (74 per cent) conceived with a service rate of 3.4 for those that became pregnant. Of 25 controls, 18 (72 per cent) conceived with a service rate of 4.2.

OVERFEEDING AND FERTILITY

It generally is believed that overfat cows have more difficulty in conceiving than do others. This belief has not been submitted to experimental test, and it may be argued that the tendency to fatten readily denotes an inherent endocrine imbalance which would, in itself, impair fertility.

There is a certain amount of supporting evidence for the idea that sterility may result from over-fatness. Marshall and Peel (28) examined the reproductive tracts of seven heifers and cows that were fat and sterile. In all of them, there were fatty deposits in the ovaries, few follicles and an unusually large amount of orange pigment in the stroma. Quinlan (41) in a series of fat cows, found that the ovaries were smaller than usual, but that they were normal except for the absence of large follicles. Newton (36) stated that in quick-fattening breeds in Argentina fatness is a frequent cause of sterility. Cows suffering from the condition had fatty deposits in the bursa ovarii which prevented its close application to the ovary. This could be detected by rectal palpation, and the cows in which it was present were not excessively fat to the eye. However, it does not follow that fat sterile cows are sterile because they are fat.

The writer considers that this review demonstrates the need for adequately controlled experiments in this field, just as much as they are needed in the

hormone and infertility field. Much, too, is to be gained if the biochemist or nutritionist works closely with the physiologist. Far too little is known regarding the manner in which feed deficiencies affect the complex functions of the reproductive organs.

REFERENCES

- (1) ALLEN, G. S. Some Observations on Stilbestrol Dipropionate in the Treatment of Anoestrus. *Vet. Record*, **55**: 168-169. 1943.
- (2) ARISTOTLE. *De Generatione Animalium*. Ed. by A. Platt. Oxford. 1910.
- (3) ASDELL, S. A., CUPPS, P. T., HARRISON, E. S., LOOSLI, J. K., SMITHCORS, J. F., AND FINCHER, M. G. Unpublished work.
- (4) AXELSON, J. Karotintillförsölns Betydelse för Nötkreaturens Fruktsamhet. *Lantmannen*, **31**: 37-39. 1947.
- (5) BARKER, C. A. V. Experience with Ascorbic Acid Therapy for Sterility in Cows. *Can. J. Comp. Med.*, **8**: 192, 195. 1944.
- (6) BAY, F., AND VOGT-MULLER, P. Continued Studies on Treatment of Sterility in Cows and Breeding Sows with Wheat Germ Oil (Vitamin E). *Vet. J.*, **90**: 288-290. 1934.
- (7) BRANTON, C., BRATTON, R. W., AND SALISBURY, G. W. Personal Communication. 1948.
- (8) CLARK, C. F. Clinical Results in the Treatment of So-called Functional Sterility in Cows. *J. Am. Vet. Med. Assoc.*, **90**: 488-492. 1937.
- (9) DAVIS, R. E., AND MADSEN, L. L. Carotene and Vitamin A in Cattle Blood Plasma with Observations on Reproductive Performance at Restricted Levels of Carotene Intake. *J. Nutrition*, **21**: 135-146. 1941.
- (10) DERIVAUX, J. Le Corps Jaune Persistant chez la Vache. Ses Rapports avec le Metabolisme Phospho-calcique. *Ann. méd. vét.*, **85**: 298-311. 1941.
- (11) DOLDER, —. Heilversuche beider Anaphrodisie des Rindes durch Injektion von Ovarial-extrakten. *Diss. Zurich*. 1926.
- (12) ECKLES, C. H. *Dairy Cattle and Milk Production*, New York. 1920.
- (13) ECKLES, C. H., BECKER, R. B., AND PALMER, L. S. A Mineral Deficiency in the Rations of Cattle. *Minn. Agr. Expt. Sta. Bull.* 229. 1926.
- (14) ECKLES, C. H., GULLICKSON, T. W., AND PALMER, L. S. Phosphorus Deficiency in the Rations of Cattle. *Minn. Agr. Expt. Sta. Tech. Bull.* 91. 1932.
- (15) ERB, R. E., ANDREWS, F. N., HAUGE, S. M., AND KING, W. A. Observations on Vitamin A Deficiency in Young Dairy Bulls. *J. Dairy Sci.*, **30**: 687-702. 1947.
- (16) GULLICKSON, T. W., PALMER, L. S., BOYD, W. L., AND OLSON, F. C. Vitamin E in the Nutrition of Cattle. *J. Dairy Sci.*, **27**: 634. 1944.
- (17) HARRIS, P. L., SWANSON, W. J., AND HICKMAN, K. C. D. Co-vitamin Studies VI. Effect of Tocopherol Supplementation on the Output of Vitamin A, Carotene and Fat by Dairy Cows. *J. Nutrition*, **33**: 411-428. 1947.
- (18) HART, E. B., HADLEY, F. B., AND HUMPHREY, G. C. The Relation of Nutrition to Contagious Cattle Abortion. *Wis. Agr. Expt. Sta. Research Bull.* 112. 1932.
- (19) HART, G. H., AND GUILBERT, H. B. Vitamin Deficiency as Related to Reproduction in Range Cattle. *Calif. Agr. Expt. Sta. Bull.* 560. 1933.
- (20) HENKE, L. A. Results of Feeding Sprouted Oats to Correct Sterility in Cattle and Swine. *J. Agr. Research*, **51**: 51-59. 1935.
- (21) HODGSON, R. E., HALL, S. R., SWEETMAN, W. J., WISEMAN, H. G., AND CONVERSE, H. T. The Effect of Vitamin A Deficiency on Reproduction in Dairy Bulls. *J. Dairy Sci.*, **29**: 669-687. 1946.
- (22) HUFFMAN, C. F. Roughage Quality and Quantity in the Dairy Ration: A Review. *J. Dairy Sci.*, **22**: 889-980. 1939.
- (23) HUFFMAN, C. F., DUNCAN, C. W., ROBINSON, C. S., AND LAMB, L. W. Phosphorus Requirement of Dairy Cattle when Alfalfa Furnishes the Principal Source of Protein. *Mich. Agr. Expt. Sta. Tech. Bull.* 134. 1933.

- (24) JONES, J. R., DOUGHERTY, R. W., AND HAAG, J. R. Relation of Nutrition to Growth and Breeding Performance in Dairy Bulls. *J. Dairy Sci.*, **28**: 311-320. 1945.
- (25) KALKUS, J. W. A Study of Goitre and Associated Conditions in Domestic Animals. *Wash. Agr. Expt. Sta. Bull.* 136. 1920.
- (26) KEITH, —. In CAMERON, A. T. *Recent Advances in Endocrinology*. 3rd Ed. Philadelphia. 1936.
- (27) KOENEN, H. Behandlung der Anaphrodisie und des Umrinderns bei Kühen und Färsen mit dem Waisenkeimöl "Evion" (E-Merek). *Vet. Med. Diss.*, Hannover. 1938. (*Animal Breeding Abs.* **9**: 16. 1941.)
- (28) MARSHALL, F. H. A., AND PEEL, W. R. "Fatness" as a Cause of Sterility. *J. Agr. Sci.*, **3**: 383-9. 1910.
- (29) McINTOSH, R. A. Breeding Problems. *Can. J. Comp. Med.* **4**: 342-347. 1940.
- (30) McINTOSH, R. A. Ascorbic Acid (Vitamin C) in the Treatment of Impotency in Bulls and Sterility in Cows. *Can. J. Comp. Med.*, **5**: 267-268. 1941.
- (31) MEIGS, E. B., AND CONVERSE, H. T. Some Effects of Different Kinds of Hay in the Ration on the Performance of Dairy Cows. *J. Dairy Sci.*, **16**: 317-328. 1933.
- (32) MILLER, F. W., AND GRAVES, R. R. Reproduction and Health Records of the Beltsville Herd of the Bureau of Dairy Industry. *USDA Tech. Bull.* 321. 1932.
- (33) MOEHL, E. Beobachtungen über die Folgen der Futtermöl bei unsern Haustieren Während der Kreigzeit. *Vet. Med. Diss.*, Bern. 1922.
- (34) MOORE, J. S. Feeding Sprouted Oats to Overcome Difficult Breeding. *Miss. Agr. Expt. Sta. Ann. Rept.*, **40**: 24. 1927.
- (35) MOUSSU, R. Sur l'Avortement épizootique des Bovidés Un Traitement Nouveau. *Rec. de Med. Vet.*, **111**: 905-919. 1935.
- (36) NEWTON, O. R. Esterilidad de la Vaca par Adiposis de los Organos Genitales. *Univ. Buenos Aires. J. Agron. Vet.*, pp. 433-444. 1939.
- (37) PALMER, L. S., FITCH, C. P., GULLICKSON, T. W., AND BOYD, W. L. Supplementary Report of an Experiment to Determine the Effect of a Low Calcium Ration on Reproduction in Cattle. *Cornell Vet.*, **25**: 229-246. 1935.
- (38) PALMER, L. S., GULLICKSON, T. W., BOYD, W. L., FITCH, C. P. AND NELSON, J. W. The Effect of Rations Deficient in Phosphorus and Protein on Ovulation, Estrus and Reproduction of Dairy Heifers. *J. Dairy Sci.*, **24**: 199-210. 1941.
- (39) PHILLIPS, P. H., LARDY, H. A., ROYER, P. D., AND WERNER, G. M. The Relationship of Ascorbic Acid to Reproduction in the Cow. *J. Dairy Sci.* **24**: 153-158. 1941.
- (40) PHILLIPS, P. H., LARDY, H. A., HEIZER, E. E., AND RUPPEL, I. W. Sperm Stimulation in the Bull Through the Subcutaneous Administration of Ascorbic Acid. *J. Dairy Sci.*, **23**: 873-878. 1940.
- (41) QUINLAN, J. Researches into Sterility of Cows in South Africa. 15th Ann. Rept. Director of Vet. Services, Union of S. Africa, Pp. 833-1055. 1929.
- (42) QUINLAN, J., ROUX, L. L., AND VAN ASWEGAN, W. G. Researches into Sterility of Cows in South Africa. *Onderstepoort J. Vet. Sci.*, **12**: 233-249. 1939.
- (43) RICHTER, J. Die Sterilität des Rinds. Berlin. 1926.
- (44) RIDDELL, W. H., HUGHES, J. S., AND FITCH, J. B. The Relation of Phosphorus Deficiency to the Utilization of Feed in Dairy Cattle. *Kansas Agr. Expt. Sta. Tech. Bull.* **36**. 1934.
- (45) RISSE, W. Behandlung der Unfruchtbarkeit mit standardisierten Weizenkeimöl (Vitamin E) und Hormovilan bei Rindern. *Diss.*, Hannover. 1926. (*Animal Breeding Abs.*, **7**: 220-221. 1939).
- (46) SCHEIDENHELM, E. C., BORTREK, A. L., HUFFMAN, C. F., AND CLARK, C. F. Some Preliminary Results of Feeding Chlorotone to Bulls. *J. Dairy Sci.*, **25**: 690-691. 1942.
- (47) SCHWEIZER, R. Ein Beitrag zur Sterilitätsbekämpfung. *Schweiz. Arch. Tierheilk.*, **87**: 374-378. 1945.

- (48) SMIRNOV-UGRJUMOV, D. N., AND LAPTEV, M. A. The Feeding of Stud Bulls During the Mating Season. *Problemy Zhivotnovod.* 2: 64-72. 1938.
- (49) SUTTON, T. S., KRAUSS, W. E., AND HANSARD, S. L. The Effect of Vitamin A Deficiency on the Young Male Bovine. *J. Dairy Sci.*, 23: 574. 1940.
- (50) THEILER, A., GREEN, H. H., AND DU TOIT, P. J. Studies in Mineral Metabolism III. Breeding of Cattle on Phosphorus Deficient Veld. *J. Agr. Sci.* 18: 369-371. 1918.
- (51) TIMIN, K. S., AND PERETURINA, M. F. The Influence of Wheat Germ on Sperm Production of Bull Sires. *Doklady Akad. Selzkhoz Nauk* 6: 17-21. 1941. (*Animal Breeding Abs.*, 11: 26. 1943).
- (52) TIMIN, K. S., AND PERETURINA, M. F. The Effect of Wheat Embryos on Sperm Production in the Bull. *Sovet. Zootch.*, 5: 96-98. 1941. (*Animal Breeding Abs.*, 11: 166. 1943).
- (53) TUFF, P. Osteomalacia and its Occurrence in Norway. *J. Comp. Path. and Therap.*, 36: 143-155. 1923.
- (54) TUTT, J. F. D. The Treatment of Sterility in Cows with Wheat Germ Oil (Vitamin E). *Vet. Record*, 49: 568-569. 1937.
- (55) VOGT-MULLER, P., AND BAY, F. On Treatment of Sterility in Cows with Wheat Germ Oil (Vitamin E). *Vet. J.*, 87: 165-170. 1931.
- (56) WALLIS, G. C. Some Effects of a Vitamin D Deficiency on Mature Dairy Cows. *J. Dairy Sci.*, 21: 315-333. 1938.
- (57) WEBSTER, W. M. Bovine Sterility in New Zealand. *Australian Vet. J.*, 8: 199-223. 1932.

THE INHERITANCE OF FUNCTIONAL CAUSES OF REPRODUCTIVE INEFFICIENCY: A REVIEW^{1, 2}

LESTER O. GILMORE³

*Minnesota Agricultural Experiment Station, Division of Dairy Husbandry,
Saint Paul, Minnesota*

That fertility is affected by the genotype of mates is borne out by observation, the opinion of popular writers in dairy cattle breeding and by research investigators. This has led to the need of paying attention to the reproductive efficiency of the family from which a prospective herd sire comes.

The great variation in the use of the terms fertility, infertility, sterility, reproductive efficiency, and others suggests the need for some delimitation of the different terms in the interest of clarity. Spielman and Jones (113) and Gregory *et al.* (37) considered the problem from its broad aspects and studied the integrated effect of all factors up to and including parturition, *i.e.* gametogenesis, estrous, ovulation, fertilization, implantation, gestation and parturition. Other workers, including Gowen (34) and Eckles (24), have used the number of services per conception as an indication of fertility. This criterion measures fertility at the earliest time possible for cattle. Unfortunately, no method is available for use with cattle to differentiate those not conceiving from failure of fertilization from those requiring extra services because of an early resorption or expulsion. From its strictest physiological sense, however, it would seem that fertility had been achieved once fertilization of the ovum by the sperm had taken place. Presumably, separate factors are involved in failure of fertilization as contrasted to the failure of subsequent processes involved in the successful completion of a gestation.

Evidence of a general nature that implicates genetic factors in fertility has been the differences found between breed groups, bull groups and cow families. K  b (62) studied the records of 7104 calvings and found that the daughters by 22 different bulls varied considerably as groups, thus indicating a genetic basis. High fertility was present in 35 groups and low fertility in 11 groups. Spielman and Jones (113) found a range in mean reproductive efficiency per cent in their breed samples of 65.55 ± 1.88 to 81.27 ± 1.67 . Between cow families, the greatest range, *i.e.* 54.46 ± 4.83 to 87.23 ± 1.62 per cent, was found within the same breed. A correlation of $\pm 0.546 \pm 0.118$ existed between the foundation cows and the mean of their respective descendants. Out of 20 cow families averaging 1.72 services per conception, Trimberger and Davis (120) reported one of extremely low fertility (2.92 s/c) and two with significantly higher fertility (1.00 and 1.22 s/c, respectively). When analyzed by bulls, the daughters of one sire required 2.25 s/c, which was significantly higher than the

¹ Paper no. 2434, Scientific Journal Series, Minnesota Agricultural Experiment Station.

² Based upon a paper presented in the symposium on Reproductive Problems of Dairy Cattle at the 43rd Annual Meeting.

³ Present address: Department of Dairy Husbandry, The Ohio State University.

mean. These observations were borne out by Lagerlöf (70), Seath and Staples (105), and Taussig (118), who found a difference in the average calving interval between daughters of different bulls. Wagener (123) made similar observations in a Brown Swiss herd. Yapp (141) found reduced fertility in certain animals of the Illini Nellie family of Brown Swiss, and the classical example of the inherited suicidal tendency of the famous Duchess Shorthorn family also exemplifies the inherited phase of infertility.

The nature of the genetic effect upon infertility is not well known. In general, from studies in laboratory and some farm animals, a reduction in fertility is thought to accompany the reduction in body size sometimes associated with inbreeding. This could be due to the general action of genes for the lack of vigor or merely the absence of genes for vigor. This, no doubt, could be a factor in cattle. Another way in which the genotype of the animal could be important is in the presence of specific genes conditioning various types of infertility. That this is the case with cattle is supported by the work of Nielsen (91), who inbred Red Danish cattle and obtained no decrease in fertility along with a reduction of fat yield, size, thrift and conformation. Kobozieff *et al.* (65), in a review of the literature, stated that inheritance may be the cause of malformations of the genitalia and of endocrine disfunction, including sterility, intersexes and hermaphrodites. The inbreeding results on Holstein-Friesians from the Bureau of Dairy Industry (136) may be interpreted in the same light. One sire, more than all others, had a low conception rate in his daughters, most of which were highly inbred. The work of other investigators indicates more specifically that at least several types of infertility are conditioned by specific gene action. Certain genes adversely affect both cows and bulls. Others are sex limited in their effect. In females, because of the severity of the abnormalities caused, some are manifested at the time of breeding for the first pregnancy. In other cases where the affliction is less the manifestation may not be detected until a later age.

FEMALE INFERTILITY

White heifer disease. This type of inherited infertility gets its name from the fact that it often is observed in white Shorthorns and perhaps also because it presumably was the affliction of the famous "white heifer that travelled." The name is non-descriptive and, therefore, unfortunate. Spriggs (114) pointed out that no single lesion is descriptive, and the commonest characteristics in the order of frequency are: (a) closed hymen or hymen persisting in varying degrees; (b) distention of one or both uterine horns, the uterine body being present in rudimentary form; (c) complete absence of cervix and anterior vagina; (d) prominence of the Wolfian ducts; (e) presence of longitudinal submucous channels in the vagina; (f) aplasia of one uterine horn. Boyd (11) suggested this form of infertility is a deficiency in the development in which the Müllerian ducts are inhibited. The difficulty usually is detected when the afflicted heifer fails to conceive. However, Boyd detected one case at 2 months of age.

The difficulties involved usually are considered adequate to prevent conception. However, in England cases of this disease have been reported by Sprigg in which conception took place although the resulting parturitions were difficult. While the majority of cases have been reported in white Shorthorns, cases have been reported in roan Shorthorns by Hart (42) and Tutt (121), red Shorthorns and Angus by Hart, Holstein-Friesians by Fincher and Williams (31), and Sellers (107), and possibly in the buffalo (93). Since in Shorthorns the number of reds and roans greatly outnumber the whites, it has been considered significant by Hutt (54) that this disease is associated most frequently with white Shorthorns. Hence, it has been concluded that linkage with a low percentage of crossing over occurs between the gene for white (*N*) and the recessive gene for this type of infertility. Its infrequent presence in other Shorthorns and in other breeds is accounted for by crossing over between the two genes on one of the autosomes. Because only females are involved, this is a case of sex-limited inheritance.

Freemartin. While the cause of this infertility undoubtedly is chemical through a humoral agent (85), it should be noted here. The primary cause is the occurrence of bisexed twinning which is fraternal or dizygotic and has an inherited basis. The weight of evidence indicating an inherited basis for multiple births of the order of twins and higher comes from studies of the repetition of multiple birth with the same cow by several workers, including Pearl (94), Hayden (43), Hutt (51) and Palumbo (92), the difference in their incidence between herds, sire groups and cow families by Johansson (58) and between breeds by a large number of workers including Jewell (57), White *et al.* (127), Lush (79), Knott (64), Johansson (58), Koch (66), Ward, (124), Bonnier (10), and Pfau *et al.* (95). The exact nature of the inheritance is unknown. Holze (46), however, claimed that twinning is due to the action of a sex-linked gene with dominant effect. Freemartins occur in approximately 91 per cent of all bisexed twins and represent those developed in the same placental membranes. The abnormal development consists essentially of an underdevelopment of the Müllerian ducts and an over development (for a female) of the parts arising from the Wolffian ducts. The degree of maldevelopment varies tremendously from no observable external freemartin characteristics to cases showing the presence of a penis extruding from the position normally occupied by the vulva. Undescended testes were palpable above the udder.

The early identification of freemartins consists of detecting a possibly abnormally large clitoris (117), underdeveloped mammary gland, a fold of skin extending from rear udder attachment to navel, and the use of an ordinary test tube to detect the size of the vagina (29). Whereas a normal heifer will offer slight resistance to the passage of this tube, in a freemartin the tube can be inserted only 3 to 3.5 inches before contacting the end of the urinogenital sinus.

Adolescent infertility. Adolescence has been considered by some (4) to be that period beginning with the first estrous and extending to the time conception is possible. This time of functional infertility has been observed in some

other forms, and if it exists in cattle it explains some of the cases of temporary sterility commonly observed. That such cases exist is supported by the observations made by Winters *et al.* (134). From matings made with heifers in their first or second estrous periods for embryological studies, a relatively large proportion of apparently abnormal ova were recovered.

The existence of this form of infertility is suggested also by the classification of the three stages of sexual development in cows by Wille (128) as follows: (a) Maturation of the hypophysis (3-6 mo.). (b) Maturation of the ovaries (6-12 mo.). In north German breeds he found a regular estrous first established between 12-15 mo. (c) Maturation of the uterus. Full maturity is not reached until completion of skeletal growth towards the end of the third year. Therefore, it was considered that abortion during the first pregnancy may occur if the cow is bred too early. Furthermore, the greater number of services per conception for cows bred for their first pregnancy in some breeds (87, 45) but not in others (6, 20) might be taken to indicate the operation of inherited factors causing adolescent infertility. No mode of inheritance is known.

Ovarian hypoplasia (underdevelopment). Gonadal hypoplasia may occur in both sexes and was found by Lagerlöf (71) and Eriksson (28) in clinical examinations of 178 herds of Swedish Mountain cattle containing approximately 6000 cattle. The proportion of left sided, right sided and double sided hypoplasia in females was 82.3, 3.3 and 14.4 per cent, respectively. In bilateral hypoplasia in the female, the whole tract is underdeveloped and the animal does not come into heat, thereby being completely infertile. The follicles were more or less underdeveloped.

Most animals of the breed trace back to two bulls, according to Eriksson, one of which is thought to have carried the gene for hypoplasia. Owing to the wide use of hypoplastic bulls, the frequency of the gene for this affliction rose to 25.6 per cent in 1935. An official slaughter program, started in 1937, reduced the frequency to 7.9 per cent in 1942. The incidence of the gene during the same period was reduced from about 0.72 to 0.40 per cent. The gene involved is believed to be an autosomal recessive with a penetrance of 0.5654 in females.

Gonadless. Some heifers are found with a virtual absence of ovaries. The ovarian tissue that was present (19-25 mo. age), possibly on only one side, resembled the ovary of a bitch. Other characteristics are the small uterus, cervix and vagina of an infantile genital tract but slightly larger than it was at birth. To all external appearances, the heifers observed were normal until breeding age was reached, at which time no heat periods were evident. Normal udder development was lacking because of the absence of the follicular and corpus luteum hormones needed for its growth. This condition was observed in New York by Fincher (29) in three daughters of one cow, each daughter being sired by a different bull. The cow had two normal daughters. One was a twin and one a full sister of two of the gonadless daughters. The cow, herself, was a double granddaughter of a famous brood cow. In the absence of additional cases, this condition appears to be inherited as an autosomal dominant.

Incompletely developed uterine horns. The typical effect in this abnormality, according to Fincher and Williams (31), is the failure of the Müllerian ducts to develop into uterine horns. The common results, therefore, were uterus unicornis, atresia of one or both horns at the base, uterus didelphis (double uterus resulting from the arrested fusion of Müllerian bodies in caudal segments resulting in two distinct uteri), a persistence of the medial walls of the Müllerian ducts at the os uteri externum (two cervixes), and a fibrous band over the cervix. Fincher and Williams (31) reported complete infertility in 56.5 per cent and virtual infertility in an additional 13 per cent of the inbred daughters of a Holstein-Friesian bull produced from his own daughters. Seven inbred sons were sold as breeders with one being returned as a non-breeder. It is assumed that the male infertility was due to a different cause and ascribable to malformed Müllerian elements which are rudimentary in the bull. Therefore, it is thought, that sex limited inheritance is involved. This appears to be a case of a single recessive autosomal gene of a sex limited nature.

Female infertility. (*a. Jersey and b. Holstein*). Regarding as fertile any cow that gave birth to a calf following a normal gestation, the workers at the

TABLE 1
The relation of infertility with inbreeding (80)

Degree of inbreeding	Fertile	Infertile	Unclassed	Incidence of infertility	Gene frequency
<i>Jersey</i>					
$f = 0.03125-0.469$	149	12	4	7.41	} 0.333
$f = 0.-0.03124$	94	3	4	3.09	
<i>Holstein-Friesian</i>					
$f = 0.03125-0.469$	67	4	1	5.63	} 0.083
$f = 0.-0.3124$	21	1	0	4.54	

California station (37, 80) studied the incidence of infertility from a qualitative standpoint. Any female, free from infectious diseases was considered infertile if she failed to produce a calf after repeated services to one or more fertile bulls. No trichomoniasis existed in the herd. The infertile females were examined by a veterinarian who classified the reproductive organs as normal or abnormal. The infertility found in Jerseys was found in females that manifested normal heat, indicating normal ovarian development as in the above cases described by Fincher and Williams (31) in Holstein-Friesians. The cases of infertility found in the California Holstein-Friesians show abnormal estrous cycles and usually complete absence of heat. Thus, the latter type is differentiated from the infertility found in the Jerseys of California and the incompletely developed uterus. Two hundred sixty-six Jersey females by 16 different bulls were classified as to fertility. Those that were inbred had an incidence of infertility twice as great as those that were non-inbred (table 1). The 94 Holstein females by two different bulls classified showed an incidence of 5.63 per cent infertility among the inbred groups as compared to 4.54 per cent among the outcrossed group.

It is concluded that each of these types of infertility is due to a separate autosomal recessive gene and that the manifestation of the character is limited to the female sex.

Tubular genitalia. In a study on reproductive efficiency at the Kentucky Agricultural Experiment Station herd, Hull *et al.* (49) found a condition of tubular genitalia to prevail in the inbred daughters of one Jersey bull. The vulva and cervix were especially constricted, resulting in difficult parturition following the first pregnancy. Surgery was resorted to in seven of the nineteen daughters calving. While this appears to be a character conditioned by inheritance that affects fertility when considered in its broadest sense, the mode of inheritance is not known.

Adiposity surrounding genitals. Newton (89) has reported that fat in the cows of quick fattening breeds such as Shorthorn, Hereford and Angus may cause infertility if it extends to the genital organs. Fatty deposits in the bursa prevent the ovary and fallopian tubes from maintaining close apposition, thus preventing ova from entering the fallopian tube. The condition can be determined by rectal palpation. Its importance impinges on dairy cows, because it sometimes is present in cows which show no outward signs of excessive fat.

MALE INFERTILITY

Various types of inherited infertility in the male have been reported. They include hypoplasia of the testis, impotentia coeundi, umbilical hernia, intersex and three different types of abnormal spermatogenesis.

Testicular hypoplasia. Normal spermatogenesis does not occur because of the underdeveloped epithelium in the seminal ducts. In cases of bilateral hypoplasia, sperm formation is absent, but the sexual desire is normal or above average. A laboratory examination of Swedish Mountain bulls by Lagerlöf (71) showed varying degrees of retardation. The clinical examinations made showed the proportion of left, right and double sided involvements to be 81.9, 3.6 and 14.6 per cent, respectively. The difference found between males and females was not significant. The evidence for this affliction being inherited was established by Eriksson (28). Impotent bulls sired a larger percentage of impotent offspring than did normal bulls. By eliminating hypoplastic bulls, slaughtering some hypoplastic cows and selecting as herd bulls those not showing hypoplasia the disturbance was reduced from 16 per cent in 1937 to 7.9 per cent in 1942. Teunissen (119) found watery semen with an abnormal sperm count. This condition is caused by the same autosomal recessive gene that causes ovarian hypoplasia, the possible difference being of a sex limiting nature. In males, the penetrance is 0.43 as compared to 0.57 in females. The presence of hypoplasia also has been reported in cattle in the U. S. by Fincher *et al.* (30).

Impotentia Coeundi. The inability to copulate in the male was found by de Groot and Numans (19) to be caused by the failure of the sigmoid curve of the penis to straighten during erection. This S-shaped curve is located behind the scrotum (110). Its formation folds back about a foot of the penis in the

retracted state. The retractor penis muscles are continuations of the suspensory ligament of the anus. They come close together and pass on either side of the penis at the ventral curve of the flexure and end 5 to 6 inches posterior to the glans.

This anomaly has been treated surgically in Holland where seven cases had been observed by 1937. Although the cure is complete, it results in disseminating the causative genes. The number of cases increased to 24 by 1941, and it was found that 22 cases with complete pedigrees included one or both of two bulls with all but one case having a normal sire. The condition is thought by de Groot and Numans (19) to be caused by an autosomal recessive gene.

Umbilical hernia. In the male an umbilical hernia may become a physical interference with the act of service, according to Williams (131), who indicated that this character definitely is associated with breeding efficiency. That umbilical hernia is inherited was suggested by Murray (88) and is indicated by the data of Warren and Atkeson (125) who observed 21 cases in males with a common ancestor in three Idaho herds of Holstein-Friesian cattle. The case of one herniated female was thought to arise from non-hereditary causes. The mode of inheritance, while not definite, appeared to be that of a sex-limited dominant gene. In the Holstein-Friesian herd at the Minnesota Agricultural Experiment Station, five cases of umbilical hernia were observed by Gilmore (32) involving two males and three females, one of which was a freemartin. The condition appeared during the first month and persisted up to slaughter (at 8 months in one male) and was present in the females at 18 months. The five cases were sired by two unrelated bulls, but close relationship existed among the dams and grandams. If a dominant gene was responsible, the penetrance was low.

Intersex. Six Brown Swiss bulls with normally developed secondary sexual characteristics were found by Yapp (140, 141) to be infertile. A histological examination of one showed the absence of functional testicles (one present in rudimentary form) and accompanying ducts. A normal female reproductive system, except for a portion of the vagina and vulva, was found. Three of the cases were by one bull, two of the three cases being full brothers.

Returned tails. This terminology was used by Blake (8) to describe sperm that are abnormal with respect to their tails being turned back past the head. A marked degree of closeness was found between the observed sperm morphology and that predicted from pedigrees showing inbreeding to a bull, Z. Bulls having Z close up in their pedigrees on both sides were eight times as likely to have returned tails as bulls not connected with Z for several generations back. That morphological characteristics of the sperm are affected by the bulls genotype is evidenced further by the New Zealand work in which it was found possible to distinguish smears made with semen from Jersey bulls as compared to Friesian or Shorthorn bulls. The criterion was the wider sperm head of the Friesian and Shorthorns as contrasted to the Jerseys.

Semen quality. Deakin (17) encountered a few bulls with apparently nor-

mal semen that had poor keeping qualities. Because ejaculates with high sperm counts tend to store better, it was suggested that there is a variation in the degree of toxicity of accessory gland secretions between bulls, and that selection of bulls for artificial insemination from strains producing semen high in sperm and "neutral" accessory gland secretions might be helpful.

Failure of spermatogenesis. That the scrotum functions as a thermo-regulator has been known for some time. The temperature within the testicles as measured by Riemerschmid and Quinlan (101) remained within the narrow range of 34.7 to 37.8° C. In certain intersubgeneric crosses, the failure of males to undergo spermatogenesis has been considered to be due to excessive temperature within the seminiferous tubules. The inheritance seems to be that for the large testicle of the European (*Taurus typicus*) and the small scrotal sac from the other parent. Crosses in which this type of infertility has appeared are bison × domestic cattle (18), yak × domestic cattle (96) and yak × zebu (142).

LETHALS

In addition to the above described inherited effects on fertility itself, i.e. conception, genes affecting the viability of the fetus prior to birth or of the organism after birth are important in the over-all reproductive efficiency. The characters reviewed here are those that are associated with premature death under conditions of ordinary environment. No attempt has been made to separate sub-lethal and semi-lethal characters.

Evidence for the existence of 26 gene-causing lethals is reviewed. The tissues primarily affected are cartilage and bone, muscle, epithelium and nerve. Fifteen come under the first class in which an attempt has been made to arrange them according to the similarities of the malformation. This is done to encourage workers to report other pertinent cases that will lead to a sharper line of delineation or to an elimination of some duplicating terms, as the case may be. An attempt is made to arrange them according to the tissue primarily affected.

Several excellent reviews and check lists are available. In the order of their publication they are those by Hutt (52), Schäper (104), Eaton (23), Lerner (74) and Shrode and Lush (109).

Achondroplasia. The bull-dog calves of the Dexter breed in Ireland and England were described by Seligman in 1904 (106), who credited German investigators with having described similar calves as early as 1860. Not then known to be hereditary, the condition was observed to be extremely marked and constant. Affected calves were described as having short vertebral columns, inguinal hernia, thick and loose skin, head with extreme brachycephaly, rounded forehead bulging over a greatly depressed nose with projected mandible, long swollen tongues, cleft palate and legs about half normal in length. An abnormal thyroid, with increased vascularity and colloid commonly absent, also was described. This thyroid upset first led to the diagnosis of this disease as a cretin. The 21 cases out of 82 births (25.6 per cent) in two herds usually were aborted

after death in the sixth to eighth month of pregnancy following a pronounced accumulation of fluid in the dam by the fourth or fifth month. Wilson (132) described the fate of the Dexter breed after establishment of separate herd books for both Dexter and Kerry cattle in 1890. He reported that 25 to 50 per cent of the offspring of Dexter parents were malformed.

A black and white calf with similar symptoms was diagnosed in Minnesota by Nickerson in 1917 (90). Attention was directed to the similarity of congenital achondroplasia in humans, and the condition in cattle accordingly was so classed. Crew (15, 16) found a definitely smaller hypophysis in the compressed surroundings and concluded that the condition was due to malfunction of the hypophysis between the second and third month of pregnancy. He, accordingly, ascribed the abnormal thyroid condition to hypofunction of the anterior pituitary. It was further concluded by him that the associated anasarca, hydrocephalus and hydramnios could be caused by a posterior lobe deficiency which he found. In discussing this work, Pugh pointed out that the bull-dog calf is a non-viable form of achondroplasia, while the pug-dog and Dachshund were viable forms in which the head and limbs, respectively, were singly achondroplastic without malformation of other parts. Crew used the term, achondroplasia, as a group name to include such anomalies as micromelia, chondritis fetalis, osteogenesis imperfecta, pseudo-chondritis, cretinoid dysplasia, micromelia chondromalacia, osteoporosis, periosteal aplasia and chondrodystrophia fetalis. Crew cited the suggested term of Kaufmann (1893), chondrodystrophy fetalis, as being more descriptive than achondroplasia, of the Dexter bull-dog condition of arrested bone formation in existing cartilage. Mörch (86) pointed out that achondroplasia (chondrodystrophia fetalis) in humans always is inherited, always dominant and is produced rather frequently by mutation. A similar condition occurs in the creeper fowl (Landauer and Dunn, 73), where the lethal period has been shown to begin with the fourth day of incubation.

Against the above historical background, four types of achondroplasia will be reviewed.

Achondroplasia 1 (chondrodystrophia fetalis). That Dexter cattle (brachycephalic and micromelic) are heterozygous for the incompletely dominant gene causing this condition and, therefore, segregate in a 1:2:1 ratio was suggested by Hutt (52). The homozygous dominant one-fourth of the population are bull-dog, one-half are heterozygous and therefore Dexters, while the recessive one-fourth are normal-legged Kerry cattle. The causative gene in cattle, as in humans, appears to mutate frequently from the normal. The same abnormality has been described in Zux-Tillertal of Austria by Adametz (1), in a white-faced calf with solid body in Michigan by Downs (22) and in Jerseys in California (81). Four cases in British Friesians, reported by Berger and Innes (7), were concluded to be of this form.

Achondroplasia 2. Wriedt (137) described a condition in the Telemark cattle of Norway similar to the bulldog calf. However, it was more compatible with life, as affected calves usually are born alive after a gestation of normal

length. With the exception of one that lived 3 months, they die within a few days because of a respiratory obstruction causing asphyxiation. The recessive gene responsible was found also in grade Jerseys by Brandt (12) and Surrarrer (116) and in Guernseys by Brandt and by Winters (133) and in Ayrshires by Brandt. Weinkopff (126) described a case in German Friesians. The 12 cases described by Carmichael (13) probably should be classed here rather than as the dominant type (achondroplasia 1) on the strength of the statements that the Nganda cattle breed true (therefore, not heterozygous), and that one affected calf lived a few hours. Apparently, it was carried full term.

In one of the few cases in cattle where phenotypically similar characters are involved, critical matings were made by T. H. Riches and reported by Punnet (97), to find out if these two types of achondroplasia are caused by the same or different genes. A heterozygous Telemark bull was mated to eight Dexter cows with a resulting 24 normal calves and one mummy being produced. The normal calves were about equally divided between those with normal legs and those with Dexter-type legs. The conclusion that the characters in the two breeds were caused by two separate single autosomal genes was confirmed by segregation in the F_2 progeny which included one Telemark and three Dexter monsters.

Achondroplasia 3. This form of achondroplasia was described in California Jerseys by Gregory *et al.* (35). It is recessive but more variable in its expression than the Telemark type and the gene involved has little, if any, effect on leg length. A lower grade achondroplasia than types 1 and 2 seems to be involved. Although they may die shortly after birth, one animal lived to be slaughtered at 14 months. In common with other types of achondroplasia, the affected calves can be distinguished at birth. The head is short with a wide cranial vault. The maxilla sometimes is shortened and the palate commonly is cleft.

Achondroplasia 4 (Micromelia). This appears to be a lower type (higher viability) than that found by the California workers, with short legs and shortened mandible the distinguishing characteristics. The existence of this particular dwarfness is based on the appearance of three affected calves in the Jaroslav breed in Russia described by Ljutikov (78). They all descended from a high producing cow found in a peasant's herd. No physical defects were noted in any of her offspring, and because of her high production a program of inbreeding to her was developed. The three cases noted resulted from different descendants of this brood cow being mated, respectively, to a son, a grandson and a half-brother of this cow presumably, Zolataja (76). All cases had short legs. The lower jaws were shortened to different degrees. In the case of a twin bull calf, the disproportion of jaw length was so great that, although it could drink, the calf could not eat grass and hay. Accordingly, death followed from a secondary cause of failure to get along in the normal environment of cattle. In the other two cases, the shortening of the lower jaws was not enough to prevent them from eating any feed eaten by normal cattle.

When compared to his normal twin-brother, the most severely afflicted calf (Zolataja \times grandson) had a ratio, $\frac{\text{body length}}{\text{distance from knee to ground}}$, that was 1.4 times the normal ratio. A plan to breed the sire of the twin bulls back to his daughters did not materialize. One breeding bull, Golden May, heterozygous for micromelia, sired 14 sons that were valuable for their production inheritance and, thus, could be the source of widespread dissemination of this gene. This is another case where the presence of a lethal gene along with genes for high production can mean its widespread dissemination, sometimes before it is detected. Micromelia has been described in chickens by Asmundson (5).

Amputated (acroteriasis congenita). The rear legs may be absent from hock or may be normal. The pelvis is normal. The forelimbs end with the arm or humerus. The elbow and fore arm are absent. The humerus and scapula (shoulder blade) are ossified to form a right angle. The upper jaw is underdeveloped and the lower jaw is almost completely absent giving a "parrot bill" appearance. Affected calves show hydrocephalus. The palate is cleft. The ears are short and unsymmetrical. The affected calves are usually dead at birth or they die shortly after birth. This condition is due to a recessive gene and has been found in Swedish Friesians by Wriedt and Mohr (139). Out of 115 offspring sired by heterozygous bulls and out of daughters by heterozygous bulls, 102 were normal and 13 were affected. This ratio is in close agreement to the expected 7:1 ratio of a character affected by a single recessive gene. A similar lethal has been reported in swine (61) and a less severe one in sheep (74).

General ankylosis. The malformation of true or bony ankylosis is the abnormal union of the bones of a joint (21). The fore legs mainly are affected. These conditions have been described for Friesians in Russia by Ruzhevsky (103), in Germany by Stang (115) and in Jerseys in the United States by Spielman *et al.* (112). The descriptions of "flexed limbs" in Gir and Indo-Brazilian zebu cattle by Veiga (122) and of "curvature of the fore limbs" by Remmers (100) are of similar or identical character. If the same gene were involved in all cases, the expression is variable and may include muscular contracture, cleft palate, flexed spine, wry neck and internal abnormalities. Affected animals are dead at birth. The large number of cases described have resulted from inbreeding programs and, therefore, the appearance of a recessive gene involvement is given.

Because of the observations both of Williams (129, 130) and Spielman *et al.* (112) that symptoms of "muscular" contracture were found in cases of ankylosis, the question is raised here as to whether or not these two characteristics are different manifestations of the same genes.

Ankylosis of lower jaw. The lower jaw is ossified at its joint (region of the condyle and coronoid process of the mandible and the zygomatic process of the temporal bone). A shortening of the jaw also is found. This is caused by a recessive gene found in the Lyngdal breed of Norway by Mohr (82).

Agnathia. This imperfect development or absence of the lower jaw was

found in one Aberdeen Angus calf, and in four Jersey males by Ely *et al.* (27). The mandible of one case carried full term consisted of osseous, soft and tooth-forming tissue so reduced in amount as to give the appearance of a jawless calf. The temporomandibular joints were ossified completely. A slight hydrocephalus was found. Wattle-like appendages 0.5×0.25 cm. were found located on the side of the head. Abortion may occur as early as 75 days. A recessive gene coming from two related herd sires is indicated. A somewhat different case without wattles was described by Lalonde (72) in Quebec cattle, but nothing was reported on its inheritance.

Impacted molars. This defect also has been called "parrot beak" by Snyder (111) because of the undershot or shortened lower jaw that results in a mouth with a flattened appearance. However, a similar term, "parrot bill" was used to describe an amputated calf and the term "parrot jaw" is listed as a defect with "slight to serious discrimination" on the score card of The Purebred Dairy Cattle Association (1943) as approved by the American Dairy Science Association. Accordingly, in judging dairy cattle, the term refers to an undershot lower jaw which is not lethal.

Impacted molars, as first described by Heizer and Hervey (44) and later by Annett (3), consists of an impaction of the premolar teeth in the mandible. The interalveolar spaces are greater than normal. Both sides are affected similarly. The normal incisor teeth are normal. The jaws are normal except for a fracture on the left side apparently caused by the force of the impaction, a feature which apparently is characteristic. Affected calves are born alive but die during the first week.

Both males and females are affected. The appearance of this character has been observed in over 100 Milking Shorthorns in Ohio and New Zealand. It has been concluded by Ranstead (98) that all affected animals trace back to the same carrier. The condition of impacted molars is caused by a single recessive gene located on an autosome, with complete dominance of its allele

Hydrocephalus. The condition described in cattle by Cole and Moore (14) is internal hydrocephalus in which the fluid is found in the cerebral ventricles upon autopsy. Contrasted to this type is external hydrocephalus, as found in swine (9), rats (47) and mice (38), in which the excess fluid is found outside the brain tissue. Internal hydrocephalus in cattle is accompanied by marked swelling and great enlargement of the ventricles of the cerebrum. The pressure developed was great enough to cause an enlargement of the cranial vault two to three times the normal size. Other abnormalities found in these calves were shortened humerus and femur bones and joints giving the appearance that pressure had been applied from the ends. The twisted femurs caused a wide pelvis that made parturition difficult. The abnormal humerus gave a twisted appearance to the fore legs. Associated with this lethal were conditions for "asymmetry" (wry face) and "jumpy" (muscular incoordination). The limited data indicated that the three characters had independent inheritance, unlikely as is the chance for the presence of these three genes in one animal. The lethal, *hydro-*

cephalus, appears to be inherited as a single recessive and was found in a herd of Holstein-Friesians in Michigan where a bull was bred back to 15 daughters. A case of non-lethal hydrocephalus in the United States sired by a "Durham" bull out of a Jersey \times Herford cow was reported by Houck (47).

Cerebral hernia. Two affected Holstein-Friesian calves were found by Shaw (108) to have an opening in the skull involving a failure of complete ossification of the frontal bones with a possible involvement of the parietal bone. The opening thus resulting, with the absence of the meninges, permits the brain to protrude, causing a hernia. Such calves may be stillborn or die shortly after birth. A similar condition has been reported in humans (Catlin Mark) (33), swine (48) and mice (63), where it is inherited as a recessive.

Short spine. The vertebral column is shortened to about half the normal number of 13 ribs and corresponding thoracic vertebrae. The remaining vertebrae and ribs are fused. Calves are either stillborn or die shortly after birth due to suffocation during difficult birth with breach presentation. One case lived 4 days. The gene responsible is inherited as a recessive and was found in the Oplandske breed of Norwegian mountain cattle by Mohr and Wriedt (84).

Missing phalanges. The missing bones in this condition, described by Johansson (59, 60) in Swedish cattle, were the first and second phalanges (pastern and coronary region, respectively). As a result, the hoof connects to metacarpal and metatarsal bones only by tendons and skin. The metacarpal and metatarsal bones were shortened considerably. Accordingly, the calf cannot stand but crawls on carpal joints and the hocks. Calves are born full-term and are otherwise normal. A recessive gene is thought to be responsible.

Mummification. This causes a shortened neck, stiffened legs and prominent joints. It is thought to be associated with muscle contracture and fetal resorption. The latter may be due to a separate gene. The majority of the mummified fetuses are aborted during the eighth month of gestation. Some that apparently died during the eighth month were carried full term. This recessive lethal was found in Danish Red cattle by Löje (cited by Lerner 74).

Lameness. The affected Danish Red calves found by Nielsen (91) were lame in the rear legs and could not stand, probably because of an affliction of the tendon and ligaments. All cases show the same symptoms. The affliction permits the calves to be born alive, but they only live 2-3 weeks under normal environment. The segregation of 294 affected calves by 39 bulls, from 213 cows, including 36 cows with two lame calves each, did not agree with single factor inheritance, although all cases were traced to a single ancestor through both sire and dam. The maldevelopment was concluded to be caused by two complimentary genes.

Muscle contracture. There is extreme rigidity of the neck and all limbs. The head is bent back. The fore and rear legs are drawn together toward the body. Parturition, accordingly, is difficult and in some instances assistance is rendered by dismembering the fetus. Calves are carried full term and die during birth or shortly afterward. The dams also may be permanently injured

or rendered sterile. This lethal is caused by a recessive gene. It has been found in Norwegian and Danish Red cattle by Mohr (82). In the United States, five cases have been described in Holstein-Friesian herds of Northern Minnesota by Hutt (53). Muscle contracture also has been reported in sheep (102) and in swine (41). The suggested relationship between this character and ankylosis is given under the description of the latter character.

Epitheliogenesis imperfecta neonatorum bovis. This lethal reported by Hadley (39) and Hadley and Cole (40) is a case of imperfect development of skin with death the primary result of septicemia which results from infecting bacteria gaining entrance through the raw surfaces of the lesions. Characteristics of the abnormality are defective formation of skin below the knees and hocks, underdeveloped claws, ears deformed by rolling of the margins and growing together and denuded muzzle and mucous membrane of the nostrils, tongue, hard palate and cheeks. Affected calves are carried to full term and are normal in size. The nature of the ear defect indicated the lesions antedated birth by several weeks. Fifty-five defective calves found in 18 herds all traced back to common Holstein-Friesian stock and experimental matings indicated its inheritance to be a single recessive. The same condition has been found by Kroon and Van Der Plank (68) in Holland Friesians herds that had contained the ancestors of the Wisconsin defectives. It was reported in Montana Brown Swiss and possibly Wisconsin Shorthorns (39), and in Jerseys by Regan *et al.* (99) and Wipprecht *et al.* (135), both of whom described the condition independently from cattle of common descent (26). Hutt and Frost (55) also have found a condition in Ayrshires resembling that of the Friesians more closely than the Jersey. These authors suggested that the breed difference found by comparing the characteristics of the abnormality in Friesians and Jerseys with Ayrshires (and the Shorthorns) may be due to different genes or to an effect on the mutant gene by the genotype in which it operates.

Fused nostrils (Abnormal skull). This lethal, causing the nasal passages to be fused, was found in the mountain breed of Croatia by Ilancic (56). The calves die at birth or shortly afterward because of respiratory difficulty. The skull was depressed abnormally between the eyes. This condition was thought by Krallinger (67) to be caused by a dominant lethal gene that is inhibited by a homozygous recessive gene. Thus, a bull heterozygous for the lethal and recessive for the inhibitor mated to cows recessive for the lethal and homozygous dominant for the inhibitor would produce approximately half abnormal calves. This conclusion of Krallinger is based on the results of Ilancic in which a bull sired 37 abnormal and 33 normal calves which is almost a 1:1 ratio.

Atresi ani. Calves with an imperforate anus are born alive and may live for several days. They do not survive surgery. Both affected males and females have been found in cattle of the Ganjam district in India by Kuppuswami (69). One hundred and five cases have been reported. A case has been observed in Minnesota Holstein-Friesians by the reviewer, but in this instance it was not established as being inherited. Similar lethals are found in swine (74).

Hypotrichosis congenita (hairless). Such calves have no hair on the body except on the muzzle, eyelids, ears, pasterns, switch, umbilicus and external genitalia. Fuzz instead of normal hair is present on the rest of the body. The hair follicles, normal in number, are under-developed and, accordingly, at birth the calf has an appearance similar to that of early embryonic development. The sweat glands, however, are over-developed in affected areas. Calves are born alive but usually die a few minutes after birth. In two reports calves lived for several months. It is caused by a recessive gene. It was found in Swedish Friesians by Mohr and Wriedt (83) and Johansson (59) and in German Friesians by Eisele (25). In the United States it was found in "Durhams" by Surrarrer (116).

Congenital spasms. Affected calves are born alive and live for a few weeks. They have intermittent spasmodic movements of head and neck, usually in a vertical plane. When forced to stand they exhibit spasms in both fore and rear legs which hampers either standing or walking. Spasms are neither initiated nor intensified by noise or shock. While they live, they appear vigorous and have good appetites. It is inherited as a recessive and was traced through 10 generations of grade Jerseys by Gregory *et al.* (36).

Congenital dropsy. This abnormality was found in Swedish Lowland cattle by Johansson (59) who quotes the earlier work of Larsson. The wide range in variation of characteristics includes the accumulation of water in the subcutaneous tissues and in the thorax and abdomen. There also is much fluid in the head and neck. Affected calves are carried from 5 months to full term with a pronounced dystocia when the gestation exceeds 200 days. When carried full term they are born alive. An affected fetus may weigh two and one half times the normal. A recessive gene is involved.

Ljutikow's lethal. This lethal was named after its discoverer (75), and results either in abortion, stillbirth or in death shortly after birth. No specific cause of death was ascribed, and no gross abnormalities were noted. It was found in 14 per cent (200) of the 1423 calves studied in the Swiss, Friesian, Allgauer and Shorthorn herds located at the Alma-Alta, Kinel, Pogrom and Timiriazee Station of the U.S.S.R. This corresponds to a 7:1 ratio expected of a recessive gene segregation where the mating of heterozygous sires to their daughters was practiced.

From the same group of herds, a total of six monsters appeared. Contrary to some reviews, no evidence for an inherited cause was established. At least three of the monsters were characterized by having short legs with underdeveloped hoofs. The affected hoofs were from a front and a diagonal rear leg. The hoof either was not cloven or only mildly so.

Sex-linked lethal 1. Andreesen (2) rests his case in Angeln cattle on a low sex ratio. The progeny of 77 female lives gave 5302 males and 6594 females. This ratio of 44.57 per cent was considered a significant deviation from the expected to establish a lethal condition occurring in the male fetus. The calving interval also was longer for females assumed to carry the gene.

Sex-linked lethal 2. Evidence for the existence of a lethal causing a statistically significant greater loss in males than females was found in the Minnesota Agricultural Experiment Station Holstein-Friesian herd by Hunsaker and Gilmore (50). Three times as many males as females were dead at birth or died within a few days after birth. The differential loss was associated with cow families and with sire lines. The validity of the comparison was enhanced by comparing offspring of a three-generation sire line thought not to carry the responsible gene, with offspring of members of two other sire lines assumed to carry the gene for the lethal character. The sires in the first line were used more or less alternately with bulls of the other two lines. The mode of sex linked or sex limited inheritance is not yet clear. A recessive gene is suspected because of the inbreeding practised. This lethal is assumed to be different from the one reported by Andreesen (2).

Sex-linked lethal 3. In a Russian Swiss herd, Ljutikow (77) found a preponderance of males in some sire lines. With a total sex-ratio of 53.7 per cent males, the offspring of six bulls (including a sire, two sons and a grandson) gave a ratio of 61.5 per cent as compared to 50.4 per cent for the get by eight other herd bulls. This preponderance of males is explained by the presence of an autosomal gene lethal in heterozygous form in female and non lethal in the male (a dominant inhibitor on the Y-chromosome). It was postulated further that YA sperm are less viable than XA sperm.

The most generally recognized long-time effective treatment is the elimination from breeding stock of the genes responsible for undesirable characters. The general distribution of undesirable genes by otherwise great sires is well illustrated by Wriedt (138), who pointed out the economic disadvantage of using bulls carrying lethal-producing genes. The importance of testing bulls suspected of carrying undesirable genes is indicated.

REFERENCES

- (1) ADAMETZ, L. Neues über den disproportiomerten Zwergwuchs (Achondroplasie) als Rassen bildende Domestikationsmutation. Zeit. f. Züchtung, 3: 125-140. 1925.
- (2) ANDREESSEN, A. Untersuchungen des zahlenmässigen Geschlechtsverhältnisses beim Angler-Bind und Prüfung des Vorliegens eines rezessiven geschlechtsgebundenen (antimaskulinen) Letalfaktors. Diss. Hanover, 1940. (abs., Deut. tierärztl. Wochschr., 49: 77. 1941.)
- (3) ANNETT, H. E. Note on a New Recessive Lethal in Cattle. J. Genetics, 37: 301-302. 1939.
- (4) ASHLEY-MONTAGU, M. F. Adolescent Sterility. Quart. Rev. Biol., 14: 13-34, 192-219. 1939.
- (5) ASMUNDSON, V. S. An Inherited Micromelia in the Domestic Fowl. J. Heredity, 33: 328-330. 1942.
- (6) BERGE, S. Fertility and "Pregnancy Percentage." (translated title.) Nord. Jordbrugsforskning, 214-224. 1942. (Abs. J. Dairy Sci., 29: A143-144. 1946.)
- (7) BERGER, J., AND INNES, J. R. M. "Bull-dog" Calves (chondrodystrophy, achondroplasia) in a Friesian Herd. Vet. Record, 60: 57-58. 1948.
- (8) BLAKE, T. A. Inheritance of Morphological Characters in the Sperm of Cattle. Nature, 155: 631. 1945.

- (9) BLUNN, C. T., AND HUGHES, E. H. Hydrocephalus in Swine. *J. Heredity*, 29: 304-308. 1938.
- (10) BONNIER, G. Studies on Monozygous Cattle Twins. *Acta Agr. Suecana*. 1: 139-146. 1946.
- (11) BOYD, W. L. A Clinical Study of "White Heifer Disease." *Cornell Vet.*, 34: 337-345. 1944.
- (12) BRANDT, G. W. Achondroplasia in Calves. *J. Heredity*, 32: 183-186. 1941.
- (13) CARMICHAEL, J. "Bull Dog" Calf in African Cattle. *Nature*, 131: 878. 1933.
- (14) COLE, C. L., AND MOORE, L. A. Hydrocephalus, a Lethal in Cattle. *J. Agr. Research*, 65: 483-491. 1942.
- (15) CREW, F. A. E. The Significance of an Achondroplasia-like Condition met with in Cattle. *Proc. Roy. Soc.*, 95B: 228-254. 1923.
- (16) CREW, F. A. E. The Bull-Dog Calf: A contribution to the Study of Achondroplasia. *Proc. Roy. Soc. Med.*, 17: 39-57. 1924.
- (17) DEAKIN, A. Field Fertility Tests and Causes of Sterility of Bulls. Dominion of Canada, Dept. Agr. mimeo. pub., 1-26. 1943.
- (18) DEAKIN, A., MUIR, G. W., SMITH, A. G., AND MACLELLAN, A. S. Hybridization of Domestic Cattle and Buffalo (*Bison Americanus*). Dominion of Can., Dept. Agr. mimeo. pub., 1-10. 1941.
- (19) DEGROOT, T., AND NUMANS, S. R. Over de Erfelijkheid der Impotentia Coeundii bij Stieren. *Tijdschr. V. Diergeneeskunde*, 71: 372-379. 1946. (Abs., *Vet. Bull.*, 17: 552. 1947; *Animal Breeding Abstracts*, 15: 24. 1947.)
- (20) DINKHAUSER, F. Mehr Milch durch früheres Zulassen der Rinder. *Forschungsdienst.*, 17: 466-470. (abs., *Animal Breeding Abstracts*, 15: 100. 1947.)
- (21) DORLAND, W. A. N. The American Illustrated Medical Dictionary. 20th Ed. W. B. Saunders Co., Philadelphia. 1941.
- (22) DOWNS, W. G. An American "Dexter Monster." *Anat. Record*, 37: 365-372. 1928.
- (23) EATON, O. N. A Summary of Lethal Characters in Animals and Man. *J. Heredity*, 28: 320-326. 1937.
- (24) ECKLES, C. H. A Study of Breeding Records of Dairy Herds. *Minn. Agr. Expt. Sta. Bull.* 258. 1929.
- (25) EISELE, F. Angeborene Haarlosigkeit bei Kälbern der Schwarzbunten Niederungszucht als Auswirkung einer Letalfaktors. *Züchtungskunde*, 11: 432-437. 1936.
- (26) EDITOR. Relationship between Hairless Calves in California and Texas herds. *J. Heredity*, 26: 355-356. 1935.
- (27) ELY, F., HULL, F. E., AND MORRISON, H. B. Agnathia, a New Bovine Lethal. *J. Heredity*, 30: 105-108. 1939.
- (28) ERIKSSON, K. Hereditary Forms of Sterility in Cattle. Hakan Ohlssons Boktryckeri, Lund, 1943.
- (29) FINCHER, M. G. Methods of Increasing Fertility in Domestic Animals. *Am. Soc. Study of Sterility Trans.*, 1-17. 1946.
- (30) FINCHER, M. G., OLAFSON, P., AND FERGUSON, J. Sterility in Bulls. *Cornell Vet.*, 32: 407-423. 1942.
- (31) FINCHER, M. G., AND WILLIAMS, W. L. Arrested Development of the Muellerian Ducts Associated with Inbreeding. *Cornell Vet.*, 16: 1-19. 1926.
- (32) GILMORE, L. O. Unpublished data. *Minn. Agr. Expt. Sta.*
- (33) GOLDSMITH, W. M. The Catlin Mark. *J. Heredity*, 13: 69-71. 1922.
- (34) GOWEN, J. W. Results of Progress on Animal Husbandry Investigations in 1917. *Maine Agr. Expt. Sta. Bull.* 274. 1918.
- (35) GREGORY, P. W., MEAD, S. W., AND REGAN, W. M. A New Type of Recessive Achondroplasia in Cattle. *J. Heredity*, 33: 317-322. 1942.
- (36) GREGORY, P. W., MEAD, S. W., AND REGAN, W. M. Heredity Congenital Lethal Spasms in Jersey Cattle. *J. Heredity*, 35: 195-200. 1944.

- (37) GREGORY, P. W., REGAN, W. M., AND MEAD, S. W. Evidence of Genes for Female Sterility in Dairy Cows. *J. Genetics*, 30: 506-517. 1945.
- (38) GRÜNEBERG, H. Two New Mutant Genes in the House Mouse. *J. Genetics*, 45: 22-28. 1943.
- (39) HADLEY, E. B. Congenital Epithelial Defects of Calves. *J. Heredity*, 18: 487-495. 1927.
- (40) HADLEY, F. B., AND COLE, L. J. Inherited Epithelial Defects in Cattle. *Wisc. Agr. Expt. Sta. Research Bull.* 86. 1928.
- (41) HALLQUIST, C. Ein Fall von Letalfaktoren beim Schwein. *Hereditas*, 18: 215-224. 1933.
- (42) HART, F. W. White Heifer Disease. *Vet. Record*, 51: 63. 1939.
- (43) HAYDEN, C. C. A Case of Twinning in Dairy Cattle. *J. Heredity*, 13: 22-24. 1922.
- (44) HEIZER, E. E., AND HERVEY, M. C. Impacted Molars—a new Lethal in Cattle. *J. Heredity*, 28: 123-128. 1937.
- (45) HILDER, R. A., FOHRMAN, M. H., AND GRAVES, R. R. Relation of Various Factors to the Breeding Efficiency of Dairy Animals and to the Sex Ratio of the Offspring. *J. Dairy Sci.*, 27: 981-992. 1944.
- (46) HOLZE, J. Zwillings—Vererbung in der Rinderzucht der Oldenburger Herdbuch-Gesellschaft. *Diss. Hanover*, 1942. (abs., *Animal Breeding Abstract*, 11: 95. 1943.)
- (47) HOUCK, J. W. Hydrocephalus in Lower Animals: Congenital Occurrence in a Calf and an Albino Rat. *Anat. Record*, 45: 83-106. 1930.
- (48) HUGHES, E. H., AND HART, H. Defective Skulls Inherited in Swine. *J. Heredity*, 25: 111-115. 1934.
- (49) HULL, F. E., DIMOCH, W. W., ELY, F., AND MORRISON, H. R. Reproductive Efficiency in Dairy Cattle. *Ky. Agr. Exp. Sta. Bull.*, 402. 1940.
- (50) HUNSAKER, L. R., AND GILMORE, L. O. Unpublished data. *Minn. Agr. Expt. Sta.*
- (51) HUTT, F. B. Bovine Quadruplets. *J. Heredity*, 21: 339-348. 1930.
- (52) HUTT, F. B. Inherited Lethal Characters in Domestic Animals. *Cornell Vet.*, 24: 1-25. 1934.
- (53) HUTT, F. B. A hereditary Lethal Muscle Contracture in Cattle. *J. Heredity*, 25: 41-46. 1934.
- (54) HUTT, F. B. Some Hereditary Abnormalities of Domestic Animals. *Cornell Vet.*, 36: 180-194. 1946.
- (55) HUTT, F. B., AND FROST, J. N. Hereditary Epithelial Defects in Ayrshire Cattle. *J. Heredity*, 39: 131-137. 1948.
- (56) ILANCIC, D. Ein neuer Letalfaktor beim Rind. *Züchtungskunde*, 15: 129-133. 1940.
- (57) JEWELL, F. M. Sex Ratios in Foetal Cattle. *Biol. Bull.*, 41: 259-271. 1921.
- (58) JOHANSSON, I. The Sex Ratio and Multiple Births in Cattle. *Z. f. Zuchtung*, 324: 183-268. 1932.
- (59) JOHANSSON, I. Variations in the Manifestation of Lethal Characters in the Swedish Breeds of Cattle. *Proc. 7th Intern. Genetic Congr.*, 144. 1939.
- (60) JOHANSSON, I. Reduced Phalanges and Curly Coat; Two Mutant Characters in Native Swedish Cattle. *Hereditas*, 28: 278-288. 1942.
- (61) JOHNSON, L. E. "Streamlined" Pigs; a new Legless Mutation. *J. Heredity*, 31: 239-242. 1940.
- (62) KÄB, E. Untersuchungen über die Vererbung von Fruchtbarkeit und Unfruchtbarkeit beim gelben Frankvieh. *Dissertation, Univ. Muenchen*, 1937.) (abs., *Animal Breeding Abstracts*, 6: 200. 1938.)
- (63) KEELER, C. E. "Parted Parietals" in Mice. *J. Heredity*, 21: 19-20, 83. 1930.
- (64) KNOTT, J. C. A Study of the Gestation Period of Holstein-Friesian Cows. *J. Dairy Sci.*, 15: 87-98. 1932.
- (65) KOBOZIEFF, N., POMRIASKINSKY-KOBOZIEFF, N. A., AND DUPONT, A. Génétique et Stérilité. *Record Méd. vét.*, 119: 49-56. 1943. (abs., *Animal Breeding Abstracts*, 13: 69. 1945.)

- (66) KOCH, W. Über die wirtschaftliche Bedeutung der Zwillingsgeburten beim Rind. Süddeutsch. Landw. Tierz., 27: 413-415. 1933. (abs., Animal Breeding Abstracts, 2: 12. 1934.)
- (67) KRALLINGER, H. E. Bemerkungen, zu der Arbeit "Ein neuer Letalfaktor beim Rinde" von D. Ilancic. Zuchtungskunde, 15: 133-134. 1940.
- (68) KROON, H. M., AND VAN DER PLANK, G. M. Enkele Sub-letalfactoren. Bij Tijdschrift voor Diergeneeskunde Huisdieren in Nederland. 58: 681-694. 1931.
- (69) KUPFUSWAMI, P. S. Some Observations on the Incidence and Inheritance of Imperforate Anus in Ganjam Calves. Indian J. Vet. Sci., 7: 305-308. 1937.
- (70) LAGERLÖF, N. Ein Beitrag zur Sterilität in der Rinderzucht. Z. f. Zuchtung., B32: 47-93. 1935. (Translated into German by Bass, E., and Bass, E., Jr.)
- (71) LAGERLÖF, N. Hypoplasia of the Genitals in Cattle as a Cause of Sterility. Proc. 5th Northern Vet. Congr., Copenhagen. 1939. (abs., Cornell Vet., 31: 92. 1941.)
- (72) LALONDE, L. M. A New Type of Bovine Agnathia. J. Heredity, 31: 80-81. 1940.
- (73) LANDAUER, N., AND DUNN, L. C. Studies on the Creeper Fowl. J. Genetics, 23: 397-413. 1930.
- (74) LERNER, M. Lethal and Sublethal Characters in Farm Animals. J. Heredity, 35: 219-224. 1944.
- (75) LJUTIKOW, K. M. The Lethal Factor of Swiss Horned Cattle. Biol. Zhur., 1: 21-50. 1932. (Translated by M. H. Haydak.)
- (76) LJUTIKOW, K. M. Breeding within the Family of the champion cow "Zolotaja." (Translated title) Probl. Zivotn., 25-29. 1934. (Abs., Animal Breeding Abstracts, 3: 369. 1935.)
- (77) LJUTIKOW, K. M. Hereditary Preponderance of Males Among the Progeny of Some Sires. (Translated title) Probl. Zivotn., 2: 49-51. 1935. (Abs., Animal Breeding Abstracts, 3: 369. 1935.)
- (78) LJUTIKOW, K. M. Achondroplastic Micromelia in Jaroslav Large Horned Cattle. Biol. Zhur., 6: 413-434. 1937. (Translated by M. H. Haydak.)
- (79) LUSH, R. H. Inheritance of Twinning. J. Heredity, 16: 273-279. 1925.
- (80) MEAD, S. W., GREGORY, P. W., AND REGAN, W. M. Deleterious Recessive Genes in Dairy Bulls Selected at Random. Genetics, 31: 574-588. 1946.
- (81) MEAD, S. W., GREGORY, P. W., AND REGAN, W. M. A Recurrent Mutation of Dominant Achondroplasia in Cattle. J. Heredity, 37: 183-188. 1946.
- (82) MOHR, O. L. Dodbringende arvefactor hos husdyr og mennesker. Naturens Verden, 14: 1-31. 1930.
- (83) MOHR, O. L., AND WRIEDT, C. Hairless, a New Recessive Lethal in Cattle. J. Genetics, 19: 315-336. 1928.
- (84) MOHR, O. L., AND WRIEDT, C. Short Spine, a New Recessive Lethal in Cattle; with a Comparison of the Skeletal Deformities in Short-spine and in Amputated Calves. J. Genetics, 22: 279-297. 1930.
- (85) MOORE, C. R. Gonad Hormones and Sex Differentiation. Am. Naturalist, 78: 97-130. 1944.
- (86) MÜRCH, E. T. Achondroplasia is Always Hereditary and is Inherited Dominantly. J. Heredity, 31: 439-444. 1940.
- (87) MORGAN, R. F., AND DAVIS, H. P. Influence of Age of Dairy Cattle and Season of the Year on the Sex Ratio of Calves and Services Required for Conception. Neb. Agr. Expt. Sta. Research Bull. 104. 1938.
- (88) MURRAY, A. J. Diseases of the Digestive Organs. Diseases of Cattle, Rev. Ed., U. S. Govt. Printing Office. 31-32. 1942.
- (89) NEWTON, O. R. Esterilidad de la vaca por adiposis de los organos genitales. J. Agron. Vet., Univ. B. Aires, 433-444. 1939. (Abs., Animal Breeding Abstracts, 11: 96-97. 1943.)
- (90) NICKERSON, W. S. Achondroplasia in a Calf with a Thymus in Place of a Thyroid. J. Lancet, 27: 7-10. 1917.

- (91) NIELSEN, J. Iagttagelser over Indov hos Husdyr. Nord. Jordbrugsforskning, 5 and 6: 204-217. 1943. (Abs., Animal Breeding Abstracts, 14: 198, 224. 1946.
- (92) PALUMBO, V. On Some Cases of Multiple Births. (Translated title.) Clin. vet. (Milano), 57: 232-235. 1934. (Abs., Animal Breeding Abstracts, 3: 372-373. 1935.)
- (93) PATNAIK, K. S. Sterility due to Imperforate Os Uterus in Bovines. Indian Vet. J., 19: 249-251. 1943.
- (94) PEARL, R. A Case of Triplet Calves with some General Considerations Regarding Multiple Gestation in Normally Uniparous Animals. Maine Agr. Expt. Sta. Rept., Bull. 204. 1912.
- (95) PFAU, K. O., BARTLETT, J. W., AND SHUART, C. E. A Study of Multiple Births in a Holstein-Friesian Herd. J. Dairy Sci., 31: 241-254. 1948.
- (96) PHILLIPS, R. W., TOLSTOY, I. A., AND JOHNSON, R. G. Yaks and Yak-cattle Hybrids in Asia. J. Heredity, 37: 163-170, 207-215. 1946.
- (97) PUNNETT, R. C. The Experiments of T. H. Riches Concerning the Production of Monsters in Cattle. J. Genetics, 32: 65-72. 1936.
- (98) RANSTEAD, J. M. The Inheritance of "Parrot-beak" in New Zealand Milking Short-horns. N. Z. J. Sci. and Tech., 28 (3): 206-216. 1937.
- (99) REGAN, W. M., MEAD, S. W., AND GREGORY, P. W. An Inherited Skin-defect in Cattle. J. Heredity, 26: 357-362. 1935.
- (100) REMMERS, H. Erblich bedingte Vordergliedmassenverkrümmung des Rindes in eines Schwartzbündzucht Nordwestdeutschlands. Diss. Tierärztl. Hochschule Hannover, 32 pp. (Abs., Animal Breeding Abstracts, 8: 124. 1948.)
- (101) RIEMERSCHMID, G., AND QUINLAN, J. Further Observations on the Scrotal Skin Temperature of the Bull, with Some Remarks on the Intra-Testicular Temperature. Onderstepoort J. Vet. Sci. and Animal Ind., 17: 123-140. 1941.
- (102) ROBERTS, J. A. F. The Inheritance of a Lethal Muscle Contracture in the Sheep. J. Genetics, 21: 57-69. 1929.
- (103) RUZHEVSKY, A. B. Ankylosis and Crooked Forelegs—A New Semilethal Hereditary Factor in Friesian Cattle (Translated title). Biol. Zhur., 7: 547. 1938.
- (104) SCHÄPER, W. Konstitutionsforschung und Krankheitsbekämpfung in der Tierzucht. Z. f. Züchtung, B35: 1-88. 1936.
- (105) SEATH, D. M., AND STAPLES, C. H. Some Factors Influencing the Reproductive Efficiency of Louisiana Herds. (abs.) J. Dairy Sci., 24: 510. 1941.
- (106) SELIGMAN, C. G. Cretinism in Calves. J. Path. and Bact., 9: 311-322. 1904.
- (107) SELLERS, A. F. Personal communication. 1948.
- (108) SHAW, A. O. A Skull-defect in Cattle. J. Heredity, 29: 319-320. 1938.
- (109) SHRODE, R. R., AND LUSH, J. L. The Genetics of Cattle. Advances in Genetics, 1: 209-261. 1947.
- (110) SISSON, S., AND GROSSMAN, J. D. The Anatomy of the Domestic Animals. 3rd Ed. Rev., W. B. Saunders Co., Philadelphia. p. 599. 1938.
- (111) SNYDER, L. H. Principles of Heredity. D. C. Heath and Co. Chicago. 80 p.p. 1935.
- (112) SPIELMAN, A. A., HILL, O. J., AND MCCULLOCH, E. C. Congenital Muscular Contracture and Ankylosis in Jersey Cattle. (abs.) J. Dairy Sci., 27: 655. 1944.
- (113) SPIELMAN, A. A., AND JONES, I. R. The Reproductive Efficiency of Dairy Cattle. J. Dairy Sci., 22: 329-334. 1939.
- (114) SPRIGGS, D. N. White Heifer Disease. Vet. Record, 58: 405, 415-418. 1946.
- (115) STANG, V. Gehäuftes vorkommen von erblicher allgemeiner Gelenkverkrümmung in Verbindung mit Gavenspalt beim Rind. Z. Tierzücht. Zuchtungsbiol., 48: 280-284. 1940.
- (116) SURRARRER, T. C. Bulldog and Hairless Calves. J. Heredity, 34: 175-178. 1943.
- (117) SWETT, W. W., MATTHEWES, C. A., AND GRAVES, R. R. Early Recognition of the Freemartin Condition in Heifers Twinborn with Bulls. J. Agr. Research, 61: 587-623. 1940.

- (118) TAUSSIG, S. The Problem of Fertility in Dairy Cattle and its Improvement through Breeding. *Inter. Rev. Agr.*, 37: 256T-261T. 1946.
- (119) TEUNISSEN, G. H. B. Steriliteit bij Stieren. *Tijdschr. Diergeneesk.*, 71: 595-608. 1946.
- (120) TRIMBURGER, G. W., AND DAVIS, H. P. Predictability of Breeding Efficiency in Dairy Cattle from their Previous Conception Rate and from their Heredity. *J. Dairy Sci.*, 28: 659-669. 1945.
- (121) TUTT, J. F. D. Letter to the Editor. *Vet. Record*, 51: 92. 1939.
- (122) VEIGA, J. S. Flexed Limbs, a Sub-lethal, Simple Recessive, Hereditary Factor in the Gir and Indo-Brazil Zebu. (Translated title.) *Rev. faculdade med. vet. S. Paulo*, 3: 101-108. 1945. (Abs., *Animal Breeding Abstracts*, 14: 224-225. 1946.)
- (123) WAGENER, H. Untersuchungen über die Trächtigkeitsdauer in einer Braunviehherde. Sterilität und Vererbung. *Deut. tierärztl. Wochschr.*, 40: 761. 1932.
- (124) WARD, A. H. Calf Wastage and Nutritional Survey. 22nd Ann. Rept. New Zealand Dairy Bd., Invista House, Wellington, N. Z. Pp. 14-21. 1946.
- (125) WARREN, T. R., AND ATKESON, F. W. Inheritance of Hernia. *J. Heredity*, 22: 345-352. 1931.
- (126) WEINKOPFF, P. Eine Hemmungsmisbildung bei einem Kalb mit Mikrognathia und Brachymelia. *Berliner tierärztl. Wochschr.*, 43: 62-63. 1927.
- (127) WHITE, G. C., RETTGER, L. E., AND McALPINE, J. G. Infectious Abortion. Conn. (Storrs) *Agr. Expt. Sta. Bull.* 123. 1924.
- (128) WILLE, R. Ueber "Frühreife" und ihre Bedeutung für die Rinderzucht. *Wochschr. Wein.* 1-3. 1944. (Abs., *Animal Breeding Abstracts* 12: 196. 1944.)
- (129) WILLIAMS, W. L. Studies in Teratology. *Cornell Vet.*, 21: 25-56. 1931.
- (130) WILLIAMS, W. L. The Problem of Teratology in Clinical Veterinary Medicine. *Cornell Vet.*, 26: 1-33. 1936.
- (131) WILLIAMS, W. L. The Diseases of the Genital Organs of Domestic Animals. 2nd Ed. Published by the Author, Ithaca, N. Y. p. 20. 1939.
- (132) WILSON, J. The Origin of the Dexter-Kerry Breed of Cattle. *Proc. Roy. Dub. Soc. N.S.* 12: 1-17. 1909.
- (133) WINTERS, L. M. *Animal Breeding*. 4th Ed. John Wiley and Sons. New York. P. 140. 1948.
- (134) WINTERS, L. M., GREEN, W. W., AND COMSTOCK, R. E. Prenatal Development of the Bovine. *Minn. Agr. Expt. Sta. Bull.* 151. 1942.
- (135) WIPPRECHT, C., AND HORLACHER, W. R. A Lethal Gene in Jersey Cattle. *J. Heredity*, 26: 363-368. 1935.
- (136) WOODWARD, T. E., AND GRAVES, R. R. Results of Inbreeding Grade Holstein-Friesian Cattle. *U. S. Dept. Agr. Tech. Bull.* 927. 1946.
- (137) WRIEDT, C. Letale Faktoren. *Zeit. Zücht., Reihe B*, 3: 223-230. 1925.
- (138) WRIEDT, C. Heredity in Livestock. MacMillan and Co., Ltd., London. Pp. 131-138. 1930.
- (139) WRIEDT, C., AND MOHR, L. Amputated, a Recessive Lethal in Cattle, with a Discussion on the Bearing of Lethal Factors on the Principles of Livestock Breeding. *J. Genetics*, 20: 187-215. 1928.
- (140) YAPP, W. W. A Case of Intersex in Dairy Cattle. (abs.) *J. Dairy Sci.*, 30: 552. 1947.
- (141) YAPP, W. W. Inheritance—A Factor in Breeding Failures. Minutes Dairy Cattle Breeding Research Council, Purebred Dairy Cattle Assoc., Sept. 12, 1947.
- (142) ZAWADOWSKY, M. M. Zebu-Yak Hybrids. *J. Heredity*, 22: 296-313. 1931.

METHODS OF APPROACHING THE PROBLEM OF INFERTILITY¹

J. F. SYKES

*Bureau of Dairy Industry, Agricultural Research Administration,
United States Department of Agriculture*

In the preceding discussions of infertility, the reader must have been impressed by the absence of concrete information which would enable dairymen to prevent or correct reproductive disturbances in cattle. While there is considerable information on the effect of genetic, pathological, nutritional and physiological factors on reproductive processes, the applicability of this information to the problem of infertility, as it is seen under farm conditions, has not been determined with any degree of certainty.

It has been shown, for instance, that several nutritional deficiencies interfere with reproduction under experimental conditions. These marked deficiencies do not occur frequently under practical conditions, however, and usually the condition of the animal indicates that its general nutrition was at fault before disturbances in reproduction are evident. How important then are the so-called marginal nutrient deficiencies as factors in infertility? This has not been determined. In the pathological field, for example, vaginitis is a condition that occurs very frequently in cattle, yet the causative organism is unknown. Moreover, neither the factors which predispose to the occurrence of vaginitis nor its actual importance as a cause of infertility have been determined. Many similar examples could be cited.

In the first place, therefore, the whole problem of infertility seems to suffer from a lack of a critical experimental approach in many instances, and a great deal of detailed experimental work will be necessary in the various fields of study before much progress will be made. The need for adequate controls already has been mentioned in the discussion by Asdell (1). Every control that can be devised should receive adequate consideration because of the multiplicity of the factors that appear to affect reproduction. It would seem particularly important to have disease-free units, in which groups of cattle are maintained free from contact with other cattle. In so far as possible, groups should be studied under comparable conditions of season, environment and management.

Another important phase in the study of infertility is the need for more specific definitions and also for more numerous and detailed criteria for evaluating the effects of experimental procedures.

Some agreement on what constitutes infertility should be reached. Many criteria, including viability of the calf after birth, parturition difficulties, and others, have been used in the past as measures of fertility. In most instances, it would seem that the essential reproductive processes are reasonably normal if a living offspring is born. The actual fecundity, i.e. the ability to produce viable ova and sperm, would seem to be an even more adequate measure of actual

¹ Based upon a paper presented in the symposium on Reproductive Problems of Dairy Cattle at the 43rd Annual Meeting.

fertility. While it may be necessary to be somewhat arbitrary, some general agreement on a definition of infertility should be reached.

In this connection, the development and general acceptance of some index of fertility should be attempted. At present reliance is placed largely on services per conception or percentage of pregnancies for evaluating fertility. This is not always satisfactory. With this criterion, it is difficult to assess the contribution of a completely infertile animal to the population being studied, and it fails to take into account those animals in which conception actually occurs but in which development of the fertilized eggs does not proceed to the point where pregnancy can be detected. An index similar to that proposed by Williams (3) and more recently used by Spielman and Jones (2) should be suitable for this purpose. With an index of this sort and by using dam and daughter comparisons or other criteria, a better measure of the ability of sires or cows to transmit good reproductive performance might be obtained. The inclusion of these data along with production data on pedigrees might be quite useful.

Lack of adequate criteria by which the effects of experimental techniques can be judged more accurately and in more detail is largely a result of insufficient basic physiological data. We have no adequate information on hormone levels in the blood of cattle nor on the effects of the various hormones on ovulation, spermatogenesis and activity and function of the various parts of the reproductive tract. Similarly, we have only scattered information on the histological and histochemical reactions of the various parts of the reproductive tract under various conditions and on the effects of these on sperm transport, fertilization, implantation and subsequent nourishment of the fetus. This state of affairs has arisen partly because of lack of suitable methods with which to attack the problem. Methods for quantitative analysis of hormones, an accurate method for the detection of ovulation, and means of following changes in the various parts of the reproductive tract are among the methods which must be developed if this basic information is to be obtained.

The specific problems on which work must be done in the various fields are very numerous. While it appears that basic physiological data are of prime importance in that progress in other fields will be restricted until means for making a detailed analysis of the responses of the reproductive organs is available, no one field of study need have absolute priority. Many of these studies could be carried concurrently. As a beginning at least, the following types of problems in the various fields of work are suggested as being among those which would give a fundamental understanding of the factors affecting reproduction.

Physiological studies

1. Development of chemical or biological methods for the quantitative estimation of hormones in the blood of cattle and of methods for determining secretion rates.
2. Detailed histological and histochemical studies on the various parts of the reproductive tract at all stages of development and under a variety of physiological conditions.

3. Studies on the factors affecting hormone levels and secretion rates, and variations in the different parts of the reproductive tract.
4. Studies on the underlying principles affecting ovulation and spermatogenesis by correlating hormone levels or other factors with these functions or by studying the responses to administered hormones.
5. The effects of changes in the reproductive tract on fertilization, implantation and subsequent nourishment of the embryo, livability and transport of sperm.
6. Studies of effect of season and environment on reproductive organs and on above reactions.

Pathological studies

Attention should probably be directed now to what are called the "non-specific" organisms and abnormalities, particularly vaginitis.

1. Surveys to determine incidence and relation to infertility.
2. Study of causative organism of vaginitis.
3. Inoculation experiments with organisms from infertile cows.
4. Studies to determine conditions which predispose to the invasion and pathogenicity of organisms.
5. Studies on the relation of heredity, nutrition, management and environment on resistance to disease.

Genetic studies

1. If it is not agreed that inheritance is an important factor in sterility, a further survey of available literature and a thorough study of existing records would help to settle this point.
2. Establish herds of high and low fertility by inbreeding or other means.
3. Studies on the above herds concerning hormone levels, disease resistance, nutritive requirements and other factors as they are related to infertility.
4. Surveys and investigations to determine whether high production is compatible with fertility and to determine the conditions which contribute to this discrepancy, if it exists.

Nutritional studies

1. Controlled studies on the effects of marginal deficiencies of specific nutrients on infertility.
2. Studies on effect of energy intake uncomplicated by specific deficiencies on reproduction.
3. Surveys of existing data and experiments to determine the effects of varying rates of growth.
4. The relation of the above factors on resistance to disease, hormone levels and other possible variables.

This work must be done largely with cattle. While experiments with laboratory animals can give important leads and, when done in conjunction with similar work with cattle, may reduce the numbers of cattle necessary for con-

clusive results, adequate experimentation with cattle must be done. Therefore, progress will be time-consuming and costly.

Organization of the work on a co-operative basis, therefore, would seem very desirable. The rearing of animals under experimental conditions and their subsequent placement on selected farms where their lifetime performance could be studied would multiply greatly the number of animals which could be studied and generally increase the number of factors which could be investigated during a given time interval. The establishment of a large central laboratory adequately staffed and financed by Federal and State funds and by the breed organizations should be considered, and inter-institution co-operation should be undertaken at every opportunity. Artificial-insemination units can play a large part in this program. These groups provide the only adequate set-up for studying the effects of various factors on reproduction in the bull. Work on this phase of the problem should be restricted largely to these groups, and every encouragement should be given to them to include a broader experimental approach in their work.

Where co-operative work is undertaken the interested parties should agree as to the priority of problems, the experimental design and the technics to be used for evaluation. Indeed, agreement on these latter items on an even wider basis and the establishment of technical guidance committees would be a highly advantageous step in the proper direction. It is only by an intensive, highly co-ordinated program that reasonably rapid progress will be made in solving the problem of infertility.

REFERENCES

- (1) ASDELL, S. A. Hormones and the Treatment of Sterility in Dairy Cattle: A Review. *J. Dairy Sci.*, 32: 45-59. 1949.
- (2) SPIELMAN, ARLESS, AND JONES, I. R. The Reproductive Efficiency of Dairy Cattle. *J. Dairy Sci.*, 22: 329-334. 1939.
- (3) WILLIAMS, W. L. A Standard for Measuring the Reproductive and Dairying Efficiency of Dairy Cattle. *Cornell Vet.*, 9: 204-213. 1919.

ASSOCIATION ANNOUNCEMENT

PAPERS FOR THE 1949 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association will be held June 21 to 23, 1949, at the University of Minnesota, University Farm, St. Paul. All members who are planning to present papers should submit the title of their paper accompanied by an abstract of not more than 200 words not later than March 15 to the chairman of the program committee of their respective section. The committee chairmen are as follows:

EXTENSION SECTION:

G. HEEBINK
West Virginia University
Morgantown, West Virginia

MANUFACTURING SECTION:

E. M. BARKER
Rochester Dairy
Rochester, Minnesota

PRODUCTION SECTION:

L. A. MOORE
Bureau of Dairy Industry
Agricultural Research Administration
Washington, D. C.

The general program committee feels that in order to make it possible for more members to participate in the program no member should present more than two papers. No title will be considered that is not accompanied by a suitable abstract. It also is hoped that more contributions will be received from senior staff members and from the laboratories of industry.

Because of the difficulties encountered in showing slides, it is recommended that each speaker distribute mimeographed copies of his data, together with a brief summary of his paper.

OFFICERS AND COMMITTEES OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

ASSOCIATION OFFICERS

G. M. TROUT, Michigan, <i>Vice President</i>	W. E. PETERSEN, Minnesota, <i>President</i>
P. B. ELLSWORTH, Ohio, <i>Acting Secretary-Treasurer</i>	F. E. NELSON, Iowa, <i>Journal Editor</i>

ASSOCIATION DIRECTORS

C. Y. CANNON, Iowa	P. H. TRACY, Illinois
H. B. HENDERSON, Georgia	P. R. ELLIKER, Oregon
S. J. BROWNELL, New York	J. H. HILTON, North Carolina
P. F. SHARP, California	

ASSOCIATION COMMITTEES

BORDEN AWARD COMMITTEES

Manufacturing

I. A. GOULD, Maryland
 B. E. GOODALE, South Carolina, *Chairman*
 H. H. SOMMER, Wisconsin

Production

GLEN SALISBURY, Illinois
 G. M. CAIRNS, Maryland, *Chairman*
 LANE MOORE, Beltsville, Maryland

RESOLUTIONS COMMITTEE

H. B. HENDERSON, Georgia
 A. J. MORRIS, Utah
 A. A. SPIELMAN, Connecticut, *Chairman*
 C. F. FOUNTAINE, Kansas
 FLOYD ARNOLD, Iowa

NOMINATING COMMITTEE

P. H. TRACY, Illinois
 E. G. HOOD, Ottawa
 WARREN GIFFORD, Arkansas, *Chairman*
 OTTO HILL, Washington
 C. G. BRADT, Cornell

PROGRAM COMMITTEE

J. B. FITCH, Minnesota
 GERALD HEEBINK, West Virginia
 P. H. TRACY, Illinois, *Chairman*
 L. A. MOORE, Beltsville, Maryland
 E. M. BARKER, Minnesota

JOURNAL MANAGEMENT COMMITTEE

P. R. ELLIKER, Oregon
 G. H. WISE, Iowa
 T. S. STUTTON, Ohio, *Chairman*
 F. E. NELSON, *ex-officio*, Iowa
 P. R. ELLSWORTH, *ex-officio*, Ohio

AUDITING COMMITTEE

H. S. WILLARD, Wyoming
 W. J. BRAKEL, Ohio, *Chairman*
 R. N. KENNEDY, Ohio

NECROLOGY COMMITTEE

DON JOSEPHSON, Pennsylvania
 D. M. SEATH, Kentucky, *Chairman*
 J. P. LAMASTER, South Carolina

HONORS COMMITTEE

FORDYCE ELY, Ohio
 J. A. NELSON, Montana, *Chairman*
 P. H. TRACY, Illinois

AMERICAN FEED MANUFACTURERS AWARD COMMITTEE

R. B. BECKER, Florida
 J. G. ARCHIBALD, Massachusetts, *Chairman*
 K. S. MORROW, New Hampshire

OFFICERS AND COMMITTEES OF THE EXTENSION SECTION

SECTION OFFICERS

C. W. REEVES, Florida
Vice-Chairman
 G. HEEBINK, West Virginia, *Chairman*
 RAY ALBRECHTSEN, New York
Secretary

SECTION COMMITTEES

Program Committee

RAY ALBRECHTSEN, New York
 H. R. SEARLES, Minnesota
 G. HEEBINK, West Virginia, *Chairman*
 C. W. REAVES, Florida

Dairy Records Committee

E. J. STALLARD, Wisconsin
 C. R. GEARHART, Pennsylvania
 J. D. BURKE, New York, *Chairman*
 BRUCE HAMILTON, Georgia

4-H Club Committee

H. G. REGAN, Missouri	H. A. WILLMAN, New York, <i>Chairman</i>
L. A. HIGGINS, Mississippi	RALPH PORTERFIELD, Maryland
	II. R. SEARLES, Minnesota

Teaching Methods Committee

L. H. RICH, Utah	HILTON BOYNTON, New Hampshire, <i>Chairman</i>
RALPH WAYNE, Minnesota	L. O. COLEBANK, Tennessee

JOINT COMMITTEES OF THE PRODUCTION AND EXTENSION SECTIONS

*Breeds Relations Committee**Chairman from Production Section*

FLOYD ARNOLD, Iowa	LYNN COPELAND, Tennessee
E. C. SCHEIDENHELM, New Jersey	

*Cattle Health Committee**Chairman from Production Section*

C. J. BRADT, New York	WILLIAM WALKER, Connecticut
JOE NAGROTTE, Pennsylvania	

*Dairy Breeding Committee**E. J. PERRY, New Jersey, Chairman*

GEORGE PARSONS, Michigan	JOE TAYLOR, Pennsylvania
--------------------------	--------------------------

*Type Classification**J. W. LINN, Kansas, Chairman*

G. G. GIBSON, Texas	N. B. NICHOLS, Washington
---------------------	---------------------------

OFFICERS AND COMMITTEES OF THE MANUFACTURING SECTION

SECTION OFFICERS

D. V. JOSEPHSON, Pennsylvania	E. M. BARKER, Minnesota, <i>Chairman</i>
<i>Vice-chairman</i>	J. H. HETRICK, Illinois
	<i>Secretary</i>

SECTION COMMITTEES

Standing Committee on Milk and Cream

BURDETT HEINEMAN, Missouri	D. V. JOSEPHSON, Pennsylvania, <i>Chairman</i>
	D. J. HANKINSON, Massachusetts

Standing Committee on Butter

D. K. HOWE, Nebraska	G. H. WILSTER, Oregon, <i>Chairman</i>
	C. JENSEN, North Dakota

Standing Committee on Dairy By products

E. C. ELTING, Washington, D. C.	H. C. TRELOGAN, Washington, D. C., <i>Chairman</i>
	B. H. WEBB, Washington, D. C.

Standing Committee on Standardization of the Tests of Dairy Alkalies and Methods of Reporting Results

D. H. JACOBSEN, Chicago, Illinois	C. W. ENGLAND, Maryland, <i>Chairman</i>
	C. M. MOORE, Ohio

Standing Committee on Products Judging

P. A. DOWNS, Nebraska	G. M. TROUT, Michigan, <i>Chairman</i>
E. O. ANDERSON, Connecticut	H. G. HERZER, Mississippi
	C. J. BABCOCK, Washington, D. C.

Standing Committee on Standardizing Methods for Conducting all Phases of Babcock Testing

L. M. LAMPERT, California	G. H. WILSTER, Oregon, <i>Chairman</i>
	D. B. ROBICHAUX, Chicago, Illinois

OFFICERS AND COMMITTEES OF THE PRODUCTION SECTION

SECTION OFFICERS

G. M. CAIRNS, Maryland <i>Vice-Chairman</i>	L. A. MOORE, Beltsville, Maryland, <i>Chairman</i> L. O. GILMORE, Ohio <i>Secretary</i>
--	---

SECTION COMMITTEES

Program Committee

G. H. WISE, Iowa	L. A. MOORE, Beltsville, Maryland, <i>Chairman</i> G. M. CAIRNS, Maryland
------------------	--

Breeds Relations Committee

D. L. FOURT, Idaho	H. A. HERMAN, Missouri, <i>Chairman</i> W. W. YAPP, Illinois
--------------------	---

Dairy Cattle Breeding Committee

D. M. SEATH, Kentucky	J. L. LUSH, Iowa, <i>Chairman</i> G. R. BARETT, Wisconsin
-----------------------	--

Dairy Cattle Health Committee

W. D. POUNDEN, Ohio	W. E. PETERSEN, Minnesota, <i>Chairman</i> R. E. HOEWOOD, Michigan
---------------------	---

Type Classification Committee

L. O. GILMORE, Ohio	W. J. TYLER, West Virginia, <i>Chairman</i> A. A. SPIELMAN, Connecticut
---------------------	--

Pasture Investigation Technique Committee

J. B. SHEPHERD, Beltsville, Maryland	R. H. LUSH, Tennessee, <i>Chairman</i> W. B. NEVENS, Illinois
--------------------------------------	--

Dairy Cattle Judging Committee

R. E. JOHNSON, Connecticut	S. M. SALISBURY, Ohio, <i>Chairman</i> D. L. FOURT, Idaho
----------------------------	--

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

FEBRUARY, 1949

NUMBER 2

THE GAS REQUIREMENTS OF MOLDS. V. THE MINIMUM OXYGEN REQUIREMENTS FOR NORMAL GROWTH AND FOR GERMINATION OF SIX MOLD CULTURES^{1, 2}

D. D. MILLER³ AND N. S. GOLDING

*Division of Dairy Husbandry, Agriculture Experiment Station,
State College of Washington, Pullman*

Most people assume that molds require abundant oxygen for growth, because molds usually are seen growing on the moist surfaces of substances. This, in a very general way, is correct but establishes no idea of the amount of oxygen required for normal growth or the amount required for germination of the many species of molds. The effect of the temperature and solubility of oxygen is even less understood.

The purpose of this study was to determine the minimum oxygen required for normal growth and the minimum oxygen required to permit germination of six pure cultures of mold in the presence of nitrogen and the almost complete absence of carbon dioxide. Since both temperature and gas pressure change the solubility of oxygen in the medium, several variations in both of these factors were included.

LITERATURE

The literature pertaining to much of the work on the gas requirements of molds has been reviewed by Golding (7, 8). The available literature on the oxygen requirements of molds is limited, with many research workers assuming that abundant oxygen is required for normal growth (5, 6, 11, 12). However, there is available work which shows that various species of molds can grow in very limited supplies of oxygen (1, 3, 16, 17, 18, 19). In several cases molds have given good growth in oxygen concentrations of less than 1 per cent (2, 4, 13, 15).

In a previous paper, Golding (9) has shown that the effect of carbon dioxide on the growth of various molds must be based on the solubility of carbon dioxide in the medium or mycelium. He also has shown that possibly a similar interpretation may have to be given to the effect of oxygen concentrations on molds.

Received for publication July 23, 1948.

¹ Published as Scientific Paper no. 784, College of Agriculture and Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

² American Dairy Association Research Grant and in cooperation with the Washington State Dairy Products Commission.

³ The experimental data in this paper are taken from a thesis submitted by the senior author in partial fulfillment for the degree of Master of Science in Agriculture, 1948.

MATERIALS AND METHODS

Cultures. The following cultures from previous studies (8, 9) were used: *Aspergillus flavus* d, *Aspergillus niger* 14, *Penicillium expansum* 69, *Oospora lactis* m, and *Penicillium roqueforti* 33. *Penicillium notatum* NRRL⁴ 832 also was used. This latter mold was purified by the usual pour plate technique (8),

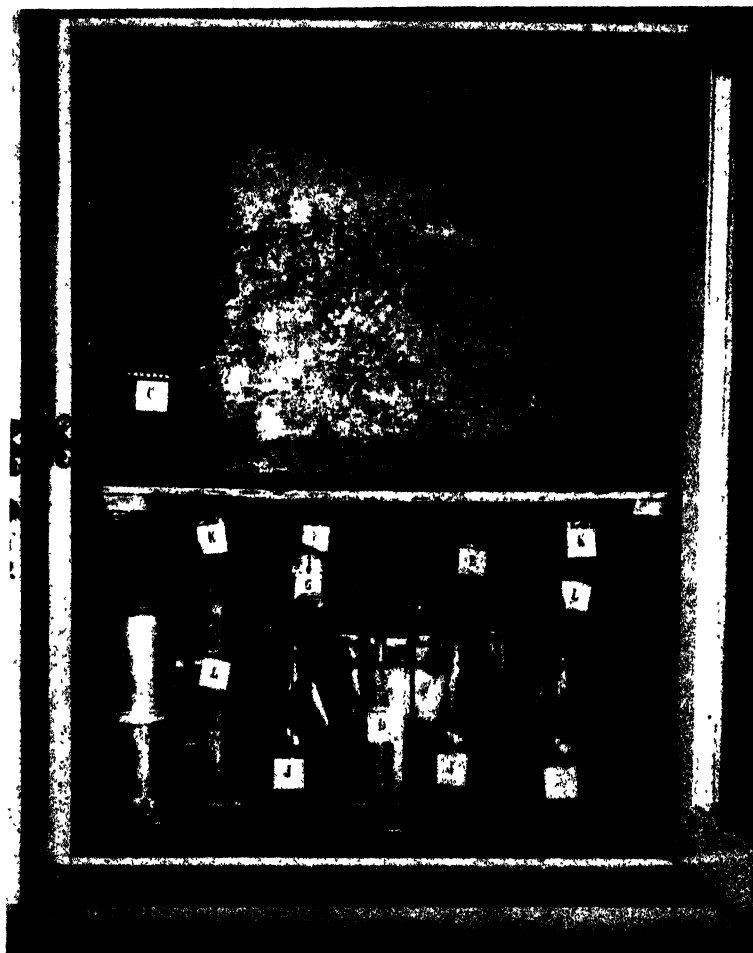


FIG. 1. The internal apparatus in each incubator.

using Difco malt agar as the medium. These species were chosen because of their wide difference in physical characteristics and temperature requirements, and because of their economic importance in food spoilage and in various manufacturing processes.

The same batch of Difco malt agar was used throughout for all plates and

⁴ Northern Regional Research Laboratory.

slants used in all experiments. The cultures of mold for inoculation of the plates were grown on malt agar slants at approximately 75° F. for 10 days. The preparation and inoculation of the plates were identical with the previous studies (7, 8, 9). After inoculation, which required approximately 1 hour, the plates were inverted, placed in their respective incubation chambers and incubated for a 7 day period.

Incubation. The same battery of incubators was used as in the previous studies (7, 8, 9). Figure 1 shows the internal apparatus in each incubator. The temperature of each incubator was controlled by a thermostat *C*. To allow either a vacuum or a pressure under which to grow the molds in the incubation chamber, 8-quart (All-American Queen) pressure cookers *D* were used, each being fitted with an inlet *E* and an outlet *F* which allowed pressure rubber

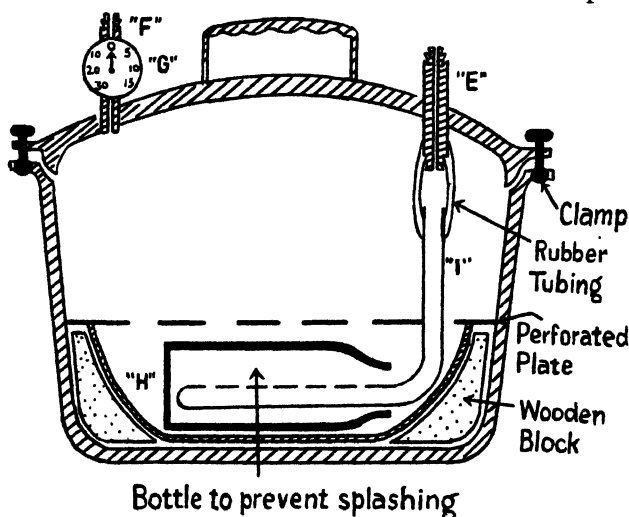


FIG. 2. A cross-section drawing of each cooker.

tubing connection. A pressure-vacuum gauge *G* was fitted to the outlet connection *F*. Figure 2 shows a cross-section drawing of each cooker *D*. In the bottom of each cooker *D*, a pyrex bowl *H* was placed containing saturated ammonium sulfate solution which controlled the humidity inside the cooker *D* at approximately 80 per cent. A similar method of humidifying has been used in previous studies (8). A perforated glass tubing *I* was so constructed as to be attached to the inlet *E* by rubber tubing, and thereby allowed the gas to bubble through the saturated ammonium sulfate solution before being a part of the gas supply of the cooker *D*.

Preceding the inlet *E*, a series of bubbling bottles *J* were placed and the gas used was bubbled continuously through 5 per cent sodium hydroxide solution to remove the carbon dioxide. Thus, the gas used contained a mixture of oxygen and nitrogen at a constant composition during the 7-day incubation period. Gas analysis established this composition.

Two tested thermometers *K* were placed one on either side of the cooker *D* and read and recorded each morning and evening. The average reading of these thermometers was considered as the temperature of incubation for the period. Each cooker was surrounded by a wooden box *L* to shield the cooker from the heating source. The slight variations in temperature from day to day seldom exceeded 2° F.

Control of gas supply. Figures 3 and 4 show the arrangement which allowed a continuous circulating system of gas supply by the use of a gas reservoir *M* and a Kelvinator refrigerator pump *N*. The gas reservoir was a 24 cubic foot cylindrical tank in which the desired oxygen concentration was ob-

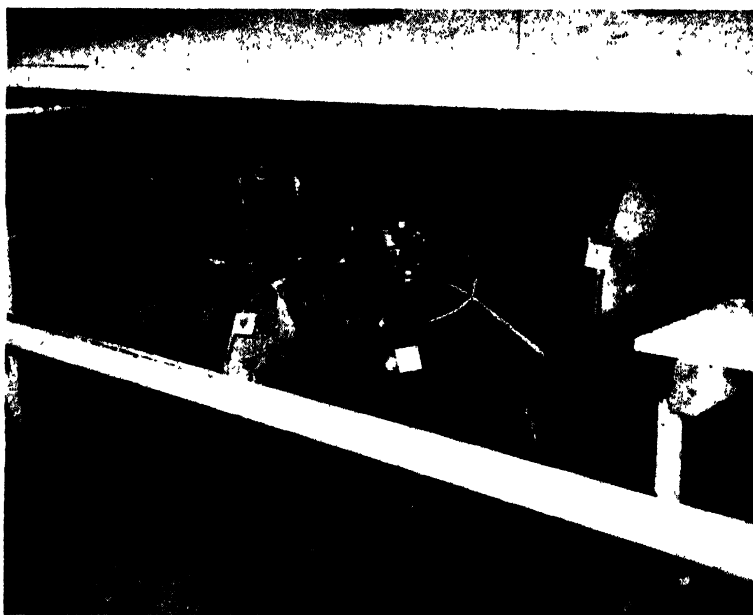


FIG. 3. Gas storage and circulation equipment.

tained by the use of air and dry nitrogen. A pressure of at least one atmosphere above the desired pressure in the cookers was maintained in this reservoir. By the use of a reducing valve *O*, the desired pressure in the cookers was regulated. Seamless copper tubing *P* carried the gas from the reservoir to the cookers and back through the refrigerator pump into the reservoir. An oil trap *Q* preceded the reservoir. This system gave a continuous flow of a desired concentration of gas over the inoculated petri dishes. To maintain the same gas concentration inside the petri dishes as was in the remainder of the cooker, it was found necessary to vary the pressure inside the cookers. This was accomplished by placing a roller *R* on a cam and gearing down so that the cam rotated once each 40 seconds. The roller cut off the gas supply by pressure on the rubber tubing which connected the reducing valve to the seamless

copper tubing. The method caused a fluctuation ranging from 10 to 20 mm. pressure above the plates each 40 seconds. Preliminary tests proved that such a condition gave more uniform growth between the same culture in different petri dishes under like conditions.

Gas analysis. The gas flowing through the system was analyzed every other day during the growth period. A Burrell-Haldane cabinet model gas analysis apparatus was used. The average of all analyses during the growth period was taken as the concentration of gases for that experiment.

Measurement of colony. The measurement of colony was identical with a previous study (8).



FIG. 4. Gas pressure control apparatus.

Expression of growth. Graphs were obtained by plotting the millimeters of growth as the ordinate and the volume of oxygen at normal temperature and pressure soluble in 1000 volumes of water as the abscissa (fig. 5). The millimeters of growth were obtained by interpolation from growth curves (14) similar to those used in previous studies (8, 9). It was assumed that the volume of oxygen soluble in the culture medium was the same as its solubility in water.

The volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water were obtained by the following formula:

$$\frac{\text{mm. pressure less vapor pressure}}{760} \times \frac{\text{partial pressure of oxygen}}{100} \times \frac{\text{oxygen absorption coefficient}}{(10)} \times 1000 = \text{volumes oxygen at N.T.P. soluble in 1000 volumes of water}$$

Example: *Oospora lactis* grown at 75° F. and 0.312 per cent oxygen showed by interpolation from growth curves a diameter of 48 mm. The average pressure during growth was 694.2 mm. mercury.

$$\text{Thus: } \frac{694.2 - 22.3}{760} \times \frac{0.312}{100} \times 0.02886 \times 1000 = 0.080 \text{ volumes oxygen at N.T.P. soluble in 1000 volumes of water}$$

EXPERIMENTAL

Figure 5 shows the effect of dissolved oxygen in water on the growth of six species of molds at various pressures. The figure shows that there is no significant difference in rate of growth between approximately 0.5, 1 and 2 atmospheres pressure, and that the effect of inhibition of growth is dependent upon the volume of oxygen dissolved in water. In air the volume at normal temperature and pressure of oxygen soluble in 1000 volumes of water ranged from 22.1 at 0.5 atmosphere and 85° F. to 132.9 at 2 atmospheres and 55° F. All six molds reached their normal growth in air at all temperatures before the volume of oxygen at normal temperature and pressure reached one part soluble in 1000 volumes of water. Therefore, for simplicity the curves are not extended beyond that concentration in this paper, but are completed in the thesis (14).

The optimum growth of the six cultures of mold were as follows: *O. lactis*, 85° F.; *P. roqueforti*, 77° F.; *A. flavus*, 90° F.; *A. niger*, 93° F.; *P. expansum*, 76° F.; *P. notatum*, 75° F. Since each culture had approximately the same break in the curve at all growing temperatures at a given oxygen solubility (a function of temperature and pressure), only the 75° F. growth curve for each is given.

O. lactis was not inhibited significantly until the oxygen supply was below 0.3 volumes soluble in 1000 volumes of water. This culture still showed growth in 7 days when the volume of oxygen soluble in 1000 volumes of water was too small to measure by analysis.

P. roqueforti was inhibited significantly when the dissolved oxygen reached approximately 0.78 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was recorded at approximately 0.08 volumes of oxygen soluble in 1000 volumes of water.

A. flavus was inhibited significantly at approximately 0.38 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained when 0 volumes of oxygen soluble in 1000 volumes of water were used.

A. niger was inhibited significantly at approximately 0.56 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained at 0.01 volumes of oxygen soluble in 1000 volumes of water.

P. expansum was inhibited significantly at approximately 0.56 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained at approximately 0.04 volumes of oxygen soluble in 1000 volumes of water.

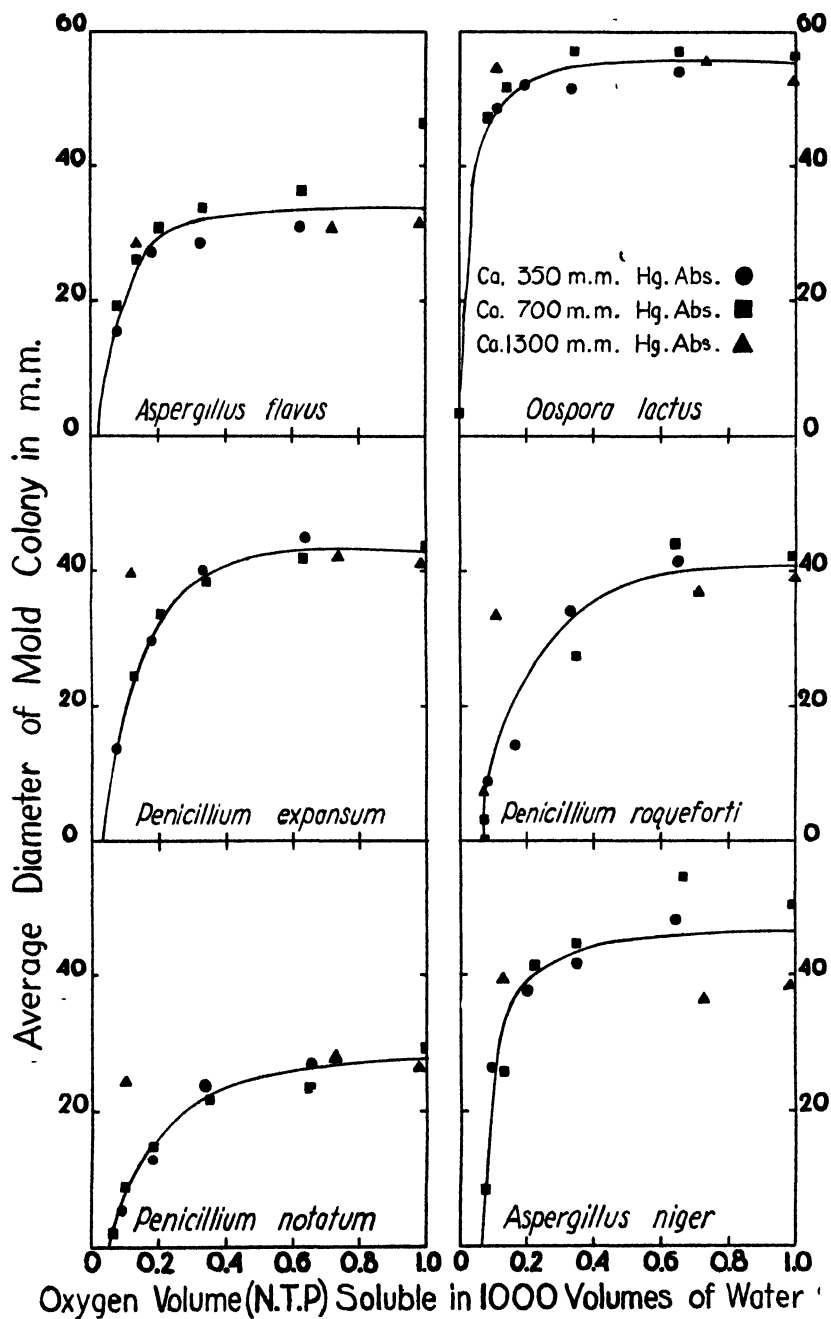


FIG. 5. The effect of dissolved oxygen on the growth of molds at 75° F.

P. notatum was inhibited significantly at approximately 0.8 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained at approximately 0.06 volumes of oxygen soluble in 1000 volumes of water.

The various curves show that the amount of dissolved oxygen required for inhibition of growth varies from *O. lactis* as the lowest to *P. roqueforti* as the highest. The sequence of the six cultures was *O. lactis*, *A. flavus*, *A. niger*, *P. expansum*, *P. notatum* and *P. roqueforti*.

DISCUSSION

From the data presented, it is shown that when the growth of molds is not restricted due to lack of oxygen, pressures of 0.5, 1 and 2 atmospheres do not significantly influence growth. It is seen that the oxygen supply must be quite low before any restriction of growth of the molds takes place. When this reduction occurs, the oxygen requirement of molds is dependent upon the dissolved gas in the medium or mycelium and not directly with the composition of oxygen in the gas above the medium. Where restriction of growth occurs due to low oxygen, the supply of this gas to the mold is influenced by the absorption coefficient and Henry's law. These data verify the previous study (9).

Workers who study the effect of oxygen and possibly other gases on mold growth at various temperatures and pressures must convert their data to terms of dissolved gases rather than using the data on the gas composition above the medium. Otherwise, the results obtained will be useful only where exactly the same temperature and atmospheric conditions exist.

The amount of dissolved oxygen used in this experiment ranged from 132.9 to 0 volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water. No appreciable effect was noted on any of the six cultures used until the volume of oxygen was below 0.8 volumes of oxygen soluble in 1000 volumes of water. Therefore, the amount of dissolved oxygen required for normal growth of various species of molds is exceedingly low.

There is a noticeable difference in the oxygen requirements of different species of molds. Of the six cultures used in this experiment, *P. roqueforti* showed the greatest amount of inhibition of growth at low volumes of dissolved oxygen. Inhibition of growth began at 0.8 volumes of oxygen soluble in 1000 volumes of water. *O. lactis*, on the other hand, did not show appreciable inhibition of growth until 0.3 volumes of oxygen soluble in 1000 volumes of water was reached. The other four cultures ranged between these extremes.

The amount of dissolved oxygen required for germination at temperatures which give growth in a 7 day period was extremely low for the cultures studied. *O. lactis* showed appreciable growth when the dissolved oxygen content was too low to measure by the Burrell-Haldane gas analysis apparatus. All cultures germinated at a dissolved oxygen content above 0.08 volumes of oxygen soluble in 1000 volumes of water.

CONCLUSIONS

1. The oxygen requirements of *O. lactis*, *P. roqueforti*, *A. flavus*, *A. niger*, *P. expansum* and *P. notatum* were studied at various percentages of oxygen

above the medium at various temperatures and pressures. It was found that the inhibiting effect of reduced oxygen is in proportion to its solubility and not directly in proportion to the composition of the gas above the medium or mycelium. Pressures between 0.5 and 2 atmospheres in air do not affect the oxygen requirements of the cultures studied.

2. No inhibitory effect on any culture was noted until the dissolved oxygen was below 0.8 volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water.

3. Complete stoppage of germination of any culture for 7 days was not obtained until the dissolved oxygen content was below 0.08 volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water. *O. lactis* showed appreciable growth when no measurable oxygen was present.

4. There is a significant difference in the oxygen requirements of different species of molds. Of the six cultures studied, *O. lactis* required the least oxygen and *P. roqueforti* the most.

REFERENCES

1. ADAMS, J., AND PARFITT, E. H. Some Factors Influencing the Amount of Mold Mycelia in Butter. *J. Dairy Sci.*, **22**, 5: 367-374. 1939.
2. BROWN, W. On the Germination and Growth of Fungi at Various Temperatures and in Various Concentrations of Oxygen and of Carbon Dioxide. *Ann. Botany*, **36** (142): 257-283. 1922.
3. EDMONDSON, J. E., AND REID, W. H. E. Various Treatments which Affect the Growth of Mold Mycelia in Cream and Resultant Butter (Abstr.). *J. Dairy Sci.*, **25**, 8: 717-718. 1942.
4. FELLOWS, H. The Influence of Oxygen and Carbon Dioxide on the Growth of *Ophiobolus graminis* in Pure Culture. *J. Agr. Research*, **37**, 6: 349-355. 1928.
5. FOSTER, J. W., WOODRUFF, H. B., AND McDANIEL, L. E. Microbiological Aspects of Penicillin. IV. Production of Penicillin in Submerged Cultures of *Penicillium notatum*. *J. Bact.*, **51**, 4: 465-478. 1946.
6. GAILEY, F. B., STEFANIAK, J. J., OLSON, B. H., AND JOHNSON, M. J. A Comparison of Penicillin-Producing Strains of *Penicillium notatum-chrysogenum*. *J. Bact.*, **52**, 1: 129-140. 1946.
7. GOLDING, N. S. The Gas Requirements of Molds. I. A Preliminary Report on the Gas Requirements of *Penicillium roqueforti* (Various Strains of Blue Mold from Cheese). *J. Dairy Sci.*, **20**, 6: 319-343. 1937.
8. GOLDING, N. S. The Gas Requirements of Molds. II. The Oxygen Requirements of *Penicillium roqueforti* (Three Strains Originally Isolated from Blue Veined Cheese) in the Presence of Nitrogen as Diluent and the Absence of Carbon Dioxide. *J. Dairy Sci.*, **23**, 9: 879-889. 1940.
9. GOLDING, N. S. The Gas Requirements of Molds. IV. A Preliminary Interpretation of the Growth Rates of Four Common Mold Cultures on the Basis of Absorbed Gases. *J. Dairy Sci.*, **28**, 10: 737-750. 1945.
10. Handbook of Chemistry and Physics. Chemical Rubber Publishing Co., Cleveland, Ohio. 30th ed. 1947.
11. HOOD, E. G., AND WHITE, A. H. Cause and Prevention of Mould in Canadian Pasteurized Butter. *Can. Dept. Agr. Bull.*, **48** (n.s.). 1925.
12. KOFFLER, H., EMERSON, R. L., PERLMAN, D., AND BURRIS, R. H. Chemical Changes in Submerged Penicillin Fermentations. *J. Bact.*, **50**, 5: 517-548. 1945.
13. MACY, H. Some of the Factors Influencing the Growth of Molds in Butter. *Minn. Agr. Expt. Sta. Tech. Bull.* **64**. 1929.

14. MILLER, D. D. The Gas Requirement of Molds. V. The Minimum Oxygen Requirements for Normal Growth and the Minimum Oxygen Requirements to Permit Germination of Six Mold Cultures. Thesis. State College of Washington, Pullman. 1948.
15. RUYLE, E. H., PEARCE, W. E., AND HAYS, G. L. Prevention of Mold in Kettled Blueberries in No. 10 Cans. Food Research, 11, 3: 274-279. 1946.
16. THOM, C., AND CURRIE, J. N. The Dominance of Roquefort Mold in Cheese. J. Biol. Chem., 15, 2: 249-258. 1913.
17. VERNON, T. R. The Deterioration of Dairy Products by Moulds. N. Z. J. Sci. and Technol., 15, 4: 237-247. 1934.
18. VERNON, T. R. Studies on the Mycological Problems of Dairying. II. The Internal and Subsurface Discoloration of Butter. J. Dairy Research, 6, 2: 168-174. 1935.
19. WILDMAN, J. D. Laboratory Studies on Development of Mold in Cream. J. Assoc. Offic. Agr. Chemists, 24, 1: 183-190. 1944.

DETERMINATION OF NITROGEN IN MILK BY DIRECT NESSLERIZATION OF THE DIGESTED SAMPLE

J. H. HETRICK AND R. M. WHITNEY¹

Dean Milk Company, Rockford, Illinois

Interest in the changes in the protein fractions present in milk due to both season and treatment made it necessary to develop a rapid method for the determination of nitrogen. The macro and even the semi-micro methods presented in the literature were not sufficiently rapid to allow for a large enough number of determinations to yield the information desired.

HISTORICAL

Since the inception of the Kjeldahl (7) method for the determination of organic nitrogen, a large amount of research has been done, broadening its application and improving its technique. It has been applied on a macro, semi-micro and micro scale in innumerable instances. An excellent review and bibliography of the work has been supplied by Bradstreet (3). This method long has been used in the determination of nitrogen in dairy products and is employed as the official method of the Association of Official Agricultural Chemists (2). As outlined in the macro scale, it is time-consuming, especially when fractionation of the organic nitrogen components of milk is desired. Rowland (10) and Menefee and Overman (9), in recognizing this, have developed semi-micro methods which have reduced considerably the time required for a determination. However, even these methods require fairly long digestion time and additional time for distillation and titration of the ammonia formed. When small amounts of ammonia are present in the sample or when micro samples are used, advantage has been taken of the Nessler reaction in place of titration. In the majority of cases (5), the Nessler reagent was added to the distillate or to a solution of ammonia obtained by aspirating air through the digested solution and into distilled water, in order to eliminate interferences present in the sample. However, in the cases of blood and urine (8), direct nesslerization of the digested mixture has been employed. Kieferle and Gloetzel (6) applied direct nesslerization to the serums obtained in the study of the action of a large number of precipitants upon the milk proteins. The work presented in this paper consists of a further investigation and modification of their technique and its application to the study of the organic nitrogen constituents of milk.

METHOD

Reagents:

- (a) NH_3 -free water: Prepare by redistilling distilled water in a 2-l. all-Pyrex glass still in the presence of 10 ml. of concentrated H_2SO_4 . The distillate

Received for publication August 20, 1948

¹ Present address: Department of Food Technology, University of Illinois, Urbana.

should be tested according to the following procedure and discarded if it contains any NH_3 , as indicated by the test.

- (b) K_2SO_4 , low nitrogen content.
- (c) H_2SO_4 , C. P., low nitrogen content.
- (d) H_2O_2 , 30 per cent.
- (e) Gum acacia solution 2 per cent w/v: Dissolve 2 g. of good-quality powdered gum acacia in NH_3 -free water in a 100 ml. volumetric flask and make up to volume. Add 1 ml. of CCl_4 as a preservative. This solution should be clear and should be discarded when turbidity develops.
- (f) Nessler's Reagent: Dissolve 50 g. of KI, C.P., in 35 ml. of cold NH_3 -free water and add a saturated solution of HgCl_2 , C.P., until the orange precipitate formed persists. Add 400 ml. of an approximately 9 N NaOH solution (360 g. of NaOH, C.P., in 1 l. of NH_3 -free water) and dilute the resultant solution to 1 l. with NH_3 -free water. This solution should be allowed to stand for several days before use, in order for equilibrium to be established.
- (g) Standard NH_4Cl stock solution (1 ml. \approx 1 mg. N): Dissolve 1.9090 g. of NH_4Cl , C.P., in NH_3 -free water and dilute to volume in a 500 ml. volumetric flask. Before using, dilute this solution further with NH_3 -free water so that 1 ml. \approx 0.01 mg. N.
- (h) $\text{K}_2\text{Cr}_2\text{O}_7$ solution 1 per cent w/v: Dissolve 1 g. of $\text{K}_2\text{Cr}_2\text{O}_7$, C.P., in distilled water and make up to 100 ml.

Apparatus:

- (a) Weight pipettes with weighing cross, if liquids are used.
- (b) Analytical balance and weights.
- (c) Micro-Kjeldahl digestion rack.
- (d) Micro-Kjeldahl flasks, calibrated to contain 50 ml., if it is desired.
- (e) Glass beads, washed with concentrated H_2SO_4 and thoroughly rinsed with NH_3 -free water.
- (f) Small measuring spoon, to contain approximately 0.4 g. of K_2SO_4 .
- (g) Spectrophotometer with accessories (Coleman-Model 11).
- (h) Standard calibrated glassware.

Permanent color blank. Transfer a measuring spoonful of K_2SO_4 , 0.5 ml. of concentrated H_2SO_4 , one glass bead and 12.5 ml. of NH_3 -free water to each of five micro-Kjeldahl flasks and digest on the rack for exactly 3 minutes after the appearance of SO_3 fumes. Cool and add three drops of 30 per cent H_2O_2 , being sure that it falls directly into the liquid in each flask. Digest for exactly 3 minutes more, cool to room temperature, dilute with NH_3 -free water, add 1 ml. of 2 per cent gum acacia solution and make up to 50 ml. with NH_3 -free water. Transfer 10 ml. aliquots to test tubes and place the tubes in a thermostat at a convenient temperature $\pm 1.0^\circ \text{C}$. (in this study the temperature selected was 23°C). Add exactly 2 ml. of Nessler's reagent to each tube and measure the percentage transmission on the spectrophotometer at $420 \text{ m}\mu$ 10 minutes after the addition of the reagent, using distilled water as a blank. These values should not deviate from each other by more than the instrumental error.

To a series of test tubes containing 10 ml. of distilled water, add 0.1, 0.2, 0.3 and 0.5 ml. of 1 per cent $K_2Cr_2O_7$ solution and measure the percentage transmission at $420 m\mu$ on the spectrophotometer, using distilled water as a blank. Plot the percentage transmission against the concentration of $K_2Cr_2O_7$ on semi-log graph paper, and from the curve obtained, determine the concentration of $K_2Cr_2O_7$ necessary to yield the same percentage transmission as the average of the reagent blanks. Figure 1 illustrates this process. Make up a $K_2Cr_2O_7$ solution of this concentration to use as a permanent color blank. The required strength of this solution will vary with the nitrogen content of the reagents used. For

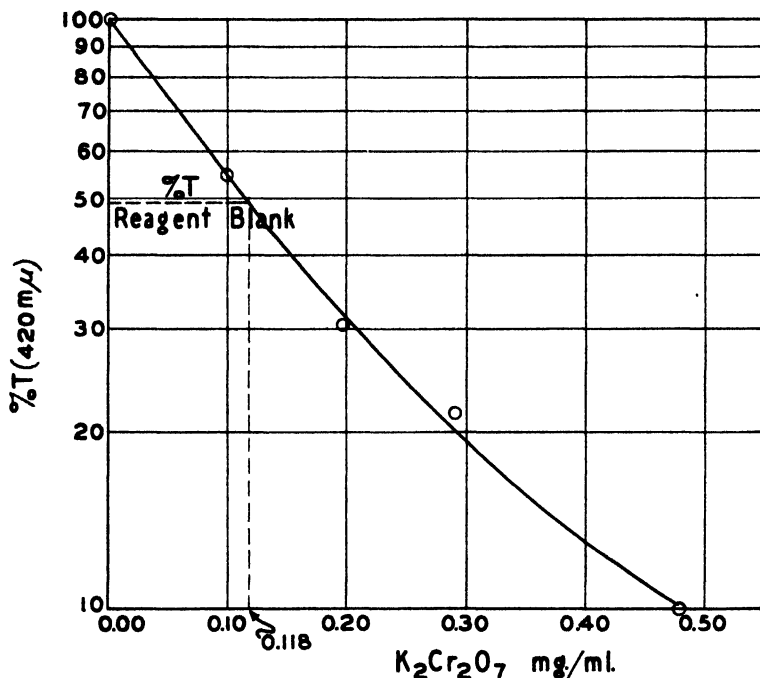


FIG. 1. Relationship between percentage transmission at $420 m\mu$ and concentration of $K_2Cr_2O_7$ solution.

comparison purposes, the solution used in this study consisted of 6.0 ml. of 1 per cent $K_2Cr_2O_7$ diluted to 500 ml.

Standard curve of percentage transmission vs. nitrogen concentration. Transfer a measuring spoonful (~ 0.4 g.) of K_2SO_4 , 0.5 ml. of concentrated H_2SO_4 and one glass bead to each of a series of ten micro-Kjeldahl flasks containing 2, 6, 8, 10, 11, 12, 13, 14, 15 and 16 ml. of the standard NH_4Cl solution (1 ml. ≈ 0.01 mg. N), and proceed as in the determination of the reagent blank with the exception that the percentage transmission is measured at $420 m\mu$ with the $K_2Cr_2O_7$ permanent color blank as a reference in place of distilled water. Plot the percentage transmission values obtained against the number of milligrams of nitrogen per 50 ml. of digested solution as indicated in figure 2

Analytical procedure. Transfer approximately 1 g. of milk, weighed accurately in a weight pipette on an analytical balance, to a 500 ml. volumetric flask and dilute to the mark with NH_3 -free water. A 12.5 ml. aliquot of this solution then is transferred to a micro-Kjeldahl flask. The procedure used in establishing the standard curve is followed except that the solution should fade from a dark-brown to a straw-yellow before the timing of the 3 minutes of additional digestion is begun. From the value obtained for the percentage transmission at $420\text{ m}\mu$ on the spectrophotometer, the amount of nitrogen present in

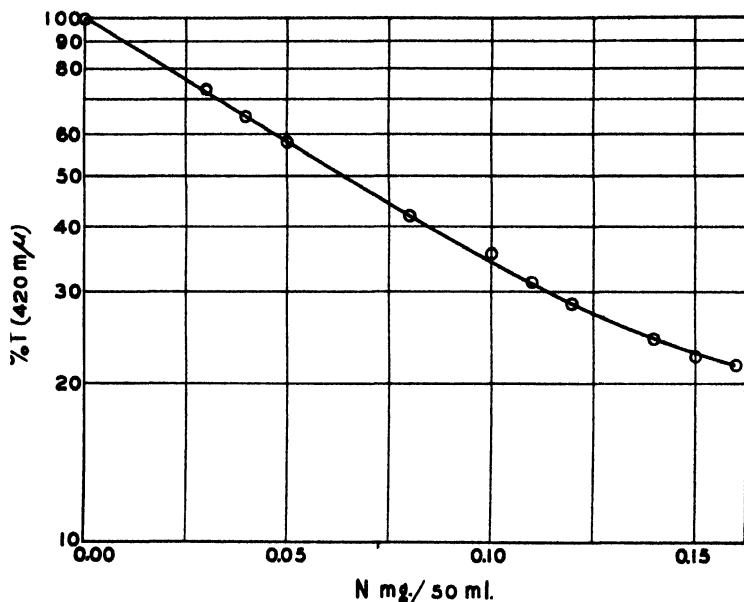


FIG. 2. Relationship between percentage transmission at $420\text{ m}\mu$ for the Nessler reagent-ammonia complex at various concentrations.

the aliquot used can be determined from the standard curve. This value in turn can be used to calculate the percentage nitrogen in the sample.

RESULTS

The Relationship Between the Wave Length and the Percentage Transmission of Light by Solutions of the Nessler Reagent-Ammonia Complex. The percentage transmission vs. the wave length curves obtained vary considerably, depending upon the particular Nessler reagent used, the amounts of various reagents present, the concentration of ammonia present, the temperature of color development and the manner of addition of the reagents. This is believed to be due to the colloidal nature of the colored complex formed. These various factors would influence the size and composition of the particles formed. No attempt was made to study these phenomena in detail, but sufficient preliminary work was done to establish the dependence of the color obtained upon the above factors. For the

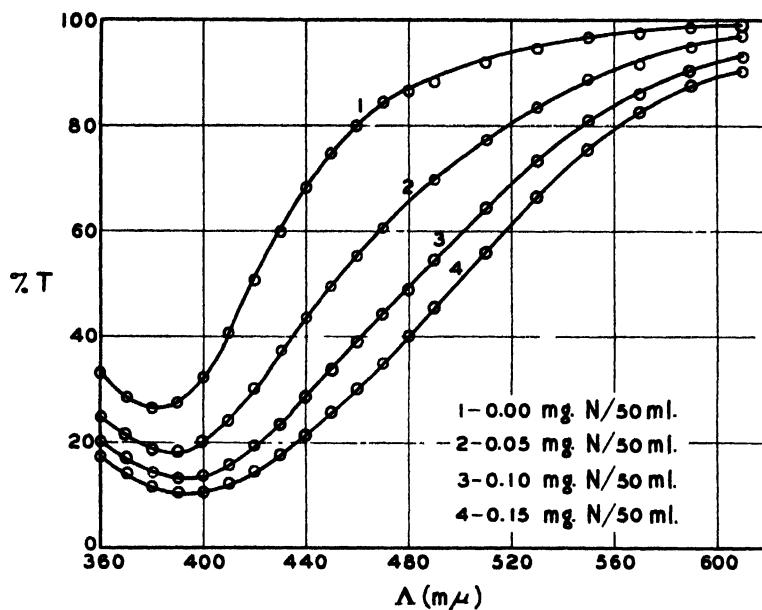


FIG. 3. Percentage transmission vs. wave length curves for the Nessler reagent ammonia complex at various concentrations.

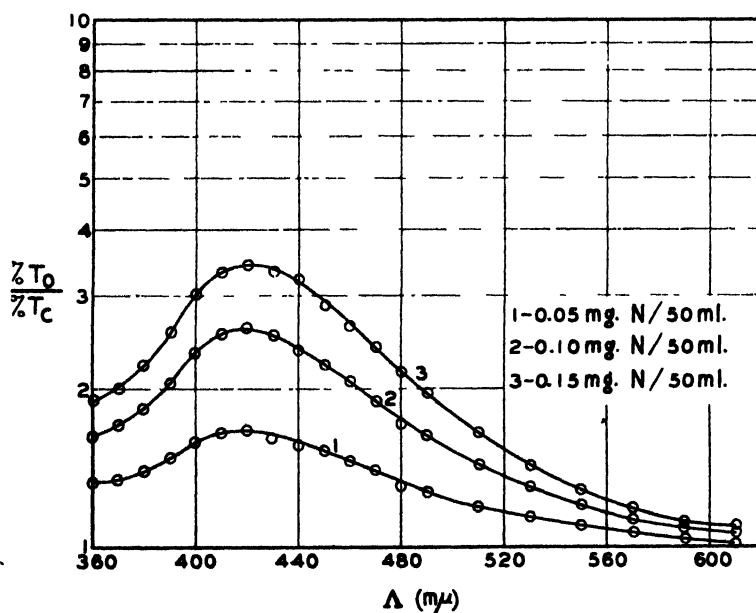


FIG. 4. $\frac{\%T_0}{\%T_c}$ vs. wave length curves for the Nessler reagent-ammonia complex at various concentrations.

particular method in this paper, the percentage transmission vs. wave length curves obtained with distilled water as a blank are given in figure 3 for several concentrations of ammonia. A decided shift in the minimum transmission toward longer wave lengths with increasing concentrations of ammonia can be noted. When this data is plotted as the logarithm of the quotient obtained by dividing the percentage transmission for the solution containing no added ammonia by the percentage transmission of the solution containing added ammonia, vs. the wave length, the curves in figure 4 are obtained. These curves all show a maximum at $420\text{ m}\mu$ which indicates the point of maximum absorption for the Nessler reagent-ammonia complex. It also may be noted that the colored complex does not follow the Bouguer-Beer-Lambert Law, since the absorption is not a linear function of the concentration. The wave length of this maximum will be a function of the Nessler reagent used and therefore, for the most accurate work, should be determined by each investigator for the Nessler reagent that he uses.

Effect of concentration of K_2SO_4 . Since Self (11) observed that ammonia

TABLE 1

Effect of concentration of K_2SO_4 upon the nitrogen values obtained by direct Nesslerization (0.9244 g. sample of milk)

Sample no.	Wt. K_2SO_4	Transmission ($420\text{ m}\mu$)	Concentration	Nitrogen
	(g.)	(%)	(mg. N/50 ml.)	(%)
1	0.0000	32.9	0.1070	0.463
2	0.1989	32.9	0.1070	0.463
3	0.4024	31.6	0.1115	0.482
4	0.6024	31.8	0.1110	0.480
5	1.0000	40.0	0.0870	0.376

was lost during digestion when the ratio of K_2SO_4 to H_2SO_4 used was favorable for the formation of the acid sulphate, it was thought wise to check the effect of variations in the concentration of K_2SO_4 used upon the amount of nitrogen obtained by this method. Therefore, five separate analyses were made on the same sample of milk with varying amounts of K_2SO_4 . The results are given in table 1.

Sample 1, to which no K_2SO_4 was added, did not become straw-yellow until after the addition of 30 per cent H_2O_2 . Sample 5 solidified in a hard cake and sputtered badly upon addition of 30 per cent H_2O_2 . These results indicate that, as long as the amount of K_2SO_4 added is not less than approximately 0.4 g. nor more than approximately 0.6 g., satisfactory results can be obtained. Therefore, the use of a measuring spoon is sufficiently accurate for the addition of K_2SO_4 . Smaller amounts should be avoided because of the difficulty in obtaining complete digestion. Larger amounts may cause loss of ammonia due to acid-sulphate formation.

Effect of concentration of H_2SO_4 . In order to determine the accuracy with which the concentrated H_2SO_4 must be measured so that it does not influence the results of the test, four complete analyses were made of the same sample of milk to which varying amounts of concentrated H_2SO_4 had been added. The results are given in table 2.

TABLE 2

Effect of concentration of H_2SO_4 upon the nitrogen values obtained by direct Nesslerization (0.9244 g. sample of milk)

Sample no.	Vol. H_2SO_4 (conc.)	Transmission (420 $m\mu$)	Concentration	Nitrogen
	(ml.)	(%)	(mg. N/50 ml.)	(%)
1	0.1	89.0	0.0112	0.048
2	0.3	32.1	0.1095	0.474
3	0.5	31.8	0.1110	0.480
4	0.7	31.9	0.1105	0.478

From these figures, it can be seen that from 0.3 to 0.7 ml. of concentrated H_2SO_4 is sufficient for complete digestion of the sample. Sample 1 behaved similarly to sample 5 in the above series.

Effect of quantity of 30 per cent H_2O_2 . To determine the limits within which the amount of H_2O_2 added must be controlled, four complete analyses were made of the same sample of milk to which varying amounts of 30 per cent H_2O_2 had been added. Table 3 shows the results of this study.

TABLE 3

Effect of quantity of 30% H_2O_2 used upon the nitrogen values obtained by direct Nesslerization (0.9244 g. sample of milk)

Sample no.	30% H_2O_2	Transmission (420 $m\mu$)	Concentration	Nitrogen
	(No. drops)	(%)	(mg. N/50 ml.)	(%)
1	0	32.0	0.1090	0.472
2	1	32.4	0.1085	0.470
3	3	32.0	0.1090	0.472
4	5	32.0	0.1090	0.472

Apparently the amount of 30 per cent H_2O_2 is not an important factor in the determination. However, since in all Kjeldahl digestion procedures encountered in the literature additional catalysts or oxidizing agents are used, it was thought desirable to retain the use of H_2O_2 as an additional precaution against incomplete digestion, even though in this sample it was not necessary.

Effect of concentration of gum acacia. When no protective colloid, such as gum acacia, is used, the ionic strength of the solution to which the Nessler reagent is added is so high that turbidity develops in the sample. In order to

TABLE 4

Effect of concentration of gum acacia upon the nitrogen values obtained by direct Nesslerization (0.9244 g. sample of milk)

Sample no.	Vol. 2% gum acacia	Transmission (420 $m\mu$)	Concentration	Nitrogen
	(ml.)	(%)	(mg. N/50 ml.)	(%)
1	0.0	31.0 (turbid)	0.1120	0.485
2	0.5	32.3	0.1085	0.470
3	1.0	32.0	0.1090	0.472
4	1.5	31.4	0.1110	0.480

check the effect of the concentration of gum acacia upon the nitrogen values obtained by the direct nesslerization method, four analyses of a sample of milk were made with additions of varying amounts of 2 per cent gum acacia solution. The results are shown in table 4.

From these values, it can be seen that 0.5 ml. or more of 2 per cent gum acacia solution is necessary in order to prevent turbidity. There is some indication, although it is within the limits of experimental error, that the gum acacia contains some free ammonia. However, slight errors in measurement of the volume of 2 per cent gum acacia will not affect the results seriously.

Effect of Nessler reagent. Several different formulae for the preparation of Nessler reagent appear in the literature (8, 6). However, only two were tested in this work. The procedure described by Kieferle and Gloetzel (6) was used first, but, after preliminary investigation, the reagent described in "The Standard Methods for Examination of Water and Sewage" (1) was selected due to its ease of preparation and sensitivity. This selection is not to be interpreted to mean that the reagent described by Kieferle and Gloetzel is not satisfactory, but merely indicates the authors' preference. The preliminary work also indicated that wide variations in the character of the color developed could be expected with variations in the concentration of Nessler reagent relative to that of the other ingredients.

Effect of temperature on color development. In preliminary work, difficulty was encountered in reproducing percentage transmission vs. concentration curves on successive days, especially when the room temperature had changed noticeably. Therefore, the effect of temperature upon the color developed was investigated with solutions of four different concentrations of NH_4Cl at three different temperatures each. Figure 5 shows the results obtained. The values for the 0.00 mg. N sample were determined with distilled water as a blank, while, for the other concentrations, the $\text{K}_2\text{Cr}_2\text{O}_7$ permanent color blank was used. Since the accuracy of temperature control was only $\pm 1^\circ \text{C.}$, no conclusions should be drawn concerning the form of the function expressing the relationship between the intensity of the color and the temperature of color development. Straight lines have been drawn connecting the values obtained as a first approximation in order to estimate the tolerance permissible in temperature control. Since the accuracy of reading the percentage transmission is ± 0.5 per cent, the temperature should be controlled to at least $\pm 1^\circ \text{C.}$

Effect of time of color development. Preliminary work indicated that the intensity of the color increases with the time of color development. In order to determine the most desirable time and the accuracy to which it should be controlled, several solutions of different concentrations of NH_4Cl were made up in the manner employed in the establishment of the standard curve and the percentage transmission measured at time intervals. Figure 6 gives the results of this study. The color appeared to increase in intensity throughout the 24-minute period during which it was followed. Therefore, 10 minutes was selected as a convenient time interval for color development. From the curves, it can

be seen that it is necessary to control this time to ± 3 minutes for the lowest concentration and to ± 6 minutes for the highest.

Reproducibility of Results and a Comparison with Values Obtained by a Modified Kjeldahl-Gunning-Arnold Method. Several analyses of two different milk samples by the method outlined in this paper and by a macro Kjeldahl method were made. The macro method used was the Kjeldahl-Gunning-Arnold

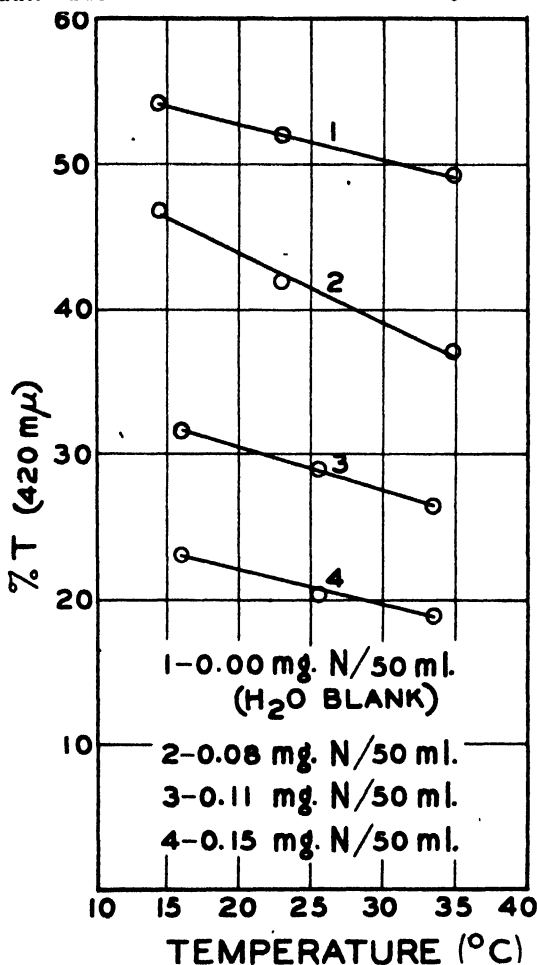


FIG. 5. Effect of temperature of color development on percentage transmission at 420 mμ of the Nessler reagent-ammonia complex at various concentrations.

method outlined in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (2) with the following modifications. Selenized granules were added as an additional catalyst in the digestion procedure, and a boric acid solution containing the mixed indicator, methyl-red and methylene blue, as described by Menefee and Overman (9), was used as a receiver for

the ammonia in place of standard 0.1 N HCl. The ammonia collected then was titrated with 0.1 N HCl to the original gray color of the indicator. These results with their standard deviations are found in table 5.

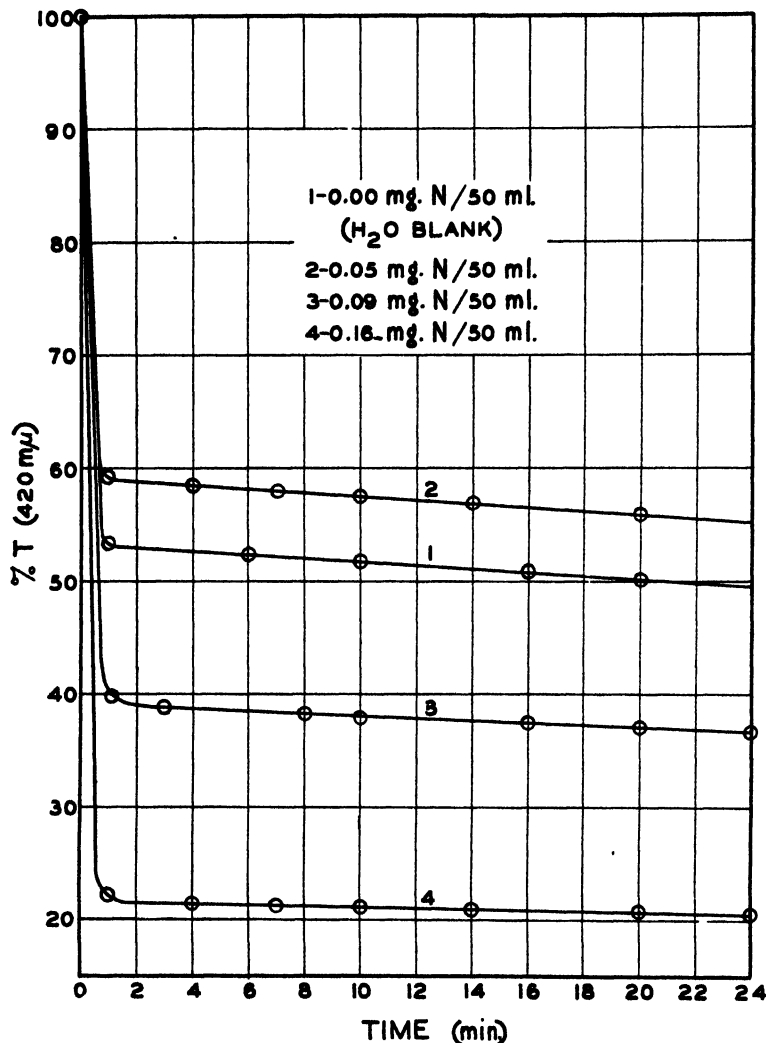


FIG. 6. Effect of time elapsing after Nesslerization on percentage transmission at 420 mμ of the Nessler reagent-ammonia complex at various concentrations.

The percentage deviation for each test from the mean of its series was calculated for the values obtained by direct nesslerization. The optimum standard deviation for these percentage deviations, defined as $\hat{\sigma} = \sqrt{\frac{\sum n_i x_i^2}{n-1}}$, was calculated to be 1.52 per cent of the mean nitrogen value. By applying the table for the χ^2

function (13) and the formula, $\chi^2 = \frac{(n-1) \hat{\sigma}^2}{\sigma^2}$, it was found that, with 90 per cent certainty, the true standard deviation, σ^2 would not be greater than ± 1.98 per cent of the mean nitrogen value. From this figure for the true standard deviation and probability tables, it is possible to predict, with 90 percent certainty, that nine times out of ten the results of a single analysis will fall within the limits of ± 3.25 per cent of the true nitrogen value of the sample. Therefore, the test may be considered sufficiently reproducible for all but the most exacting work.

When compared to the results secured by the modified Kjeldahl-Gunning-

TABLE 5

Comparison of direct Nesslerization method with modified Kjeldahl-Gunning-Arnold method for the determination of nitrogen in the same milk sample

Sample no.	Analysis no.	Direct Nesslerization		Macro Kjeldahl	
		Nitrogen	Deviation	Nitrogen	Deviation
		(%)		(%)	
1	1	0.491	-0.007	0.512	+0.0005
	2	0.490	-0.008	0.511	-0.0005
	3	0.508	+0.010		
	4	0.510	+0.012		
	5	0.495	-0.003		
	6	0.508	+0.010		
	7	0.495	-0.003		
	8	0.505	+0.007		
	9	0.495	-0.003		
	10	0.480	-0.018		
	Mean	0.498	± 0.008	0.512	± 0.0005
	Standard deviation	± 0.009			
2	1	0.482	+0.007	0.491	+0.005
	2	0.480	+0.005	0.481	-0.004
	3	0.472	-0.003	0.486	-0.000
	4	0.472	-0.003		
	5	0.475	0.000		
	6	0.471	-0.004		
	7	0.475	0.000		
	8	0.475	0.000		
	Mean	0.475	± 0.003	0.486	± 0.003
	Standard deviation	± 0.004			

Arnold method, direct nesslerization yields results which are approximately 2.5 per cent low, on the average. By employing the Student's "t" function,

$t = \frac{(\bar{X}_1 - \bar{X}_2)}{\sqrt{n_1 s_1^2 + n_2 s_2^2}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$, and consulting appropriate tables (12), this observed

difference is found to be significant. However, this deviation is within the experimental error of a single determination and, therefore, would not constitute a serious objection to the method. No explanation for this deviation has been established as yet, but Erdmann (4) has observed that some of the nitrogen present in certain organic materials is converted to the amines instead of to

ammonia. These amines would be titrated as ammonia in the Kjeldahl-Gunning-Arnold method, but would not develop the same color with the Nessler reagent. This may account for the discrepancy between the results of the two methods.

SUMMARY

A rapid method for the determination of nitrogen in milk by direct nesslerization of digested sample has been described.

The effect of the variables involved in the test has been investigated and their tolerance established.

When compared with a modified Kjeldahl-Gunning-Arnold method for the macro determination of nitrogen, the method yields results which are approximately 2.5 per cent low on the average, but are within the experimental error.

The method yields reproducible results which are sufficiently accurate for all but the most exacting work, with a considerable saving in time and reagents when compared with other methods currently in use.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the aid of Mr. Joseph Tobias, Department of Food Technology of the University of Illinois, in confirming some of the data presented.

REFERENCES

1. AMERICAN PUBLIC HEALTH ASSOCIATION AND AMERICAN WATER WORKS ASSOCIATION. Standard Methods for the Examination of Water and Sewage, 9th Ed. New York, N. Y. P. 65. 1946.
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 6th Ed. Washington, D. C. Pp. 26-28. 1945.
3. BRADSTREET, B. B. A Review of the Kjeldahl Determination of Organic Nitrogen. Chem. Rev., 27: 331-350. 1940.
4. ERDMANN, C. C. On Alkylamines as Products of the Kjeldahl Digestion. J. Biol. Chem., 8: 41-55. 1910.
5. FOLIN, O., AND FARMER, C. J. A New Method for the Determination of Total Nitrogen in Urine. J. Biol. Chem., 11: 493-501. 1912.
6. KIEFERLE, F., AND GLOETZL, J. Beitrage zur Kenntnis des Reststickstoffs der Kuhmilch. Milchw. Forsch., 11: 62-118. 1930.
7. KJELDAHL, J. En ny Methode til Kvaestofbestemmelse i organiske Stoffer. Medd. Carlsberg Lab., 2: 1-27. 1883.
8. KOCH, F. C., AND McMEEKIN, T. L. A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Folin Reagent for Ammonia. J. Am. Chem. Soc., 46: 2066-2069. 1924.
9. MENEFEE, S. G., AND OVERMAN, O. B. A Semi-micro-Kjeldahl Method for the Determination of Total Nitrogen in Milk. J. Dairy Sci., 23: 1177-1185. 1940.
10. ROWLAND, S. J. The Determination of the Nitrogen Distribution in Milk. J. Dairy Research, 9: 42-46. 1938.
11. SELF, P. A. W. An Unrecognized Source of Error in the Kjeldahl-Gunning Method for the Determination of Nitrogen. Pharm. J., 88: 384-385. 1912.
12. TEELOAR, A. E. An Outline of Biometric Analysis. Burgess Publishing Co., Minneapolis, Minn. Pp. 146-152. 1936.
13. WORTHING, A. G., AND GEFFNER, J. Treatment of Experimental Data. 1st Ed. John Wiley & Sons, Inc., New York, N. Y. P. 320. 1943.

SOME PHYSICAL AND CHEMICAL PROPERTIES OF BOVINE SALIVA WHICH MAY AFFECT RUMEN DIGESTION AND SYNTHESIS¹

J. T. REID² AND C. F. HUFFMAN

Institute of Nutrition and Dairy Department, Michigan State College

The early studies of Pavlov (25) placed great significance on the role of saliva mainly as that of a watery secretion. Although it generally is accepted that the high water content of saliva is a digestive aid as a lubricant, solvent and medium, other constituents and properties of saliva appear to be of equal importance in rumen digestion. Doubtless some of these substances and properties exert an influence on the activity of rumen flora and fauna.

Scheunert and Trautmann (30) found that the water content of parotid and mandibular saliva of sheep ranged from 98.75 to 98.90 per cent and 99.0 to 99.6 per cent, respectively. The submaxillary saliva of the cow (19) and the parotid saliva of the buffalo (33) were found to contain 99.11 and 99.15 per cent water, respectively.

The following figures were reported for the pH of saliva of several ruminant species: buffalo, 8.8 (33); sheep, 8.12 to 8.32 (28); goats, 8.2 to 8.8 (2); calves, 8.1 (32, 38) and 8.23 (31)¹; and cattle, 8.55 to 8.90 with an average of 8.8 (10). Chrzaszcz and Schechtlówna (10) observed a decrease in the pH of saliva to 7.9 during rumination.

Some reducing properties and several associated constituents of saliva have been investigated by several workers. Suzuki (36) reported that human saliva generally contains less than 0.18 mg. per cent ascorbic acid, and that age and sex are without effect upon the concentration of this substance. This investigator was not able to detect ascorbic acid in oxidized form in human saliva or in the salivary glands of rabbits. The ascorbic acid content of the salivary glands was found to be progressively lower in the species of the following orders: rodentia, carnivora and ungulata (36). Suzuki (36) demonstrated an increase in the ascorbic acid content of the parotid glands of the rabbit by injecting 2 mg. of ascorbic acid per kg. of body weight or by administering pilocarpine hydrochloride; the latter substance, however, did not alter the concentration in the submaxillary glands. The investigations of Zimmet and Dubois-Ferriere (40) revealed that the concentration of ascorbic acid in children's saliva increased progressively with age. Levels of 0.04 mg. per cent for 4-year-old children, 0.11 mg. per cent for 16-year-olds, and a marked lowering of the saliva ascorbic acid level in children under febrile conditions were reported (40). These results, relative to the age effects, are contrary to those of Suzuki (36). Held *et al.* (14) believed 0.12 to 0.16 mg. per cent to be the normal ascorbic acid

Received for publication August 23, 1948.

¹ Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal article no. 951 (n.s.).

² Present address: Department of Animal Husbandry, Cornell University, Ithaca, New York.

content of human saliva. Concentrations of 0.04 to 0.08 mg. per cent were found in the saliva of patients suffering from parodontoses (14). A higher normal level (0.25 mg. per cent) was reported by Stuteville (35). Suzuki (37) showed that the glutathione content of the parotid and submaxillary glands of scorbutic guinea pigs was 12.2 to 26.2 per cent and 16.5 to 17.4 per cent lower, respectively, than those of normal guinea pigs. Pilocarpine decreased the glutathione content of these glands in rabbits, whereas atropine was without effect. Injections of iodoacetic acid or sodium iodoacetate resulted in the complete disappearance of reduced glutathione from these glands. Pincus (26, 27) demonstrated the reduction of several dyes by saliva.

Very little attention has been given to the physical or physico-chemical properties of saliva. However, in his study of surface energy of some physiological fluids, Wwedensky (39) found that the lowering of surface tension by saliva was considerable and classified it with bile and milk, as a surface active fluid. In a later study of surface activity of human saliva, Borissovsky and Wwedensky (6) found that the secretion stimulated by acids manifested a surface tension-temperature curve characteristic of surface inactive fluids, whereas a curve characteristic of surface active substances was demonstrable for saliva stimulated by food material. These studies suggest that saliva evoked by acid solutions is a protective reaction, while that produced as a result of food excitation is a normal secretion. Simultaneous studies conducted on the relative adsorption by the salivas produced by these two methods of stimulation showed typical adsorption by the food-stimulated saliva, whereas adsorption curves characteristic of surface inactive substances were obtained for the acid-stimulated secretion.

Fomin (13) reported that the saliva of calves varies considerably in viscosity and that the phenomenon appears attributable to the consumption of different types of feed. Hoepfner (15) demonstrated a pronounced structure-viscosity in human saliva which persisted for 24 hours despite definite chemical changes. Curves which are characteristic of structure-viscous liquids were obtained in this investigation by plotting the differential flow-velocity against time. Fresh saliva was converted completely into foam and had a foaming capacity equivalent to that of a 0.02 per cent saponin solution.

Recognition of literature has been limited here to reports dealing primarily with the saliva of ruminant animals and to studies of saliva in other animals and human beings which are pertinent to certain phases of the study summarized in this report.

The object of this investigation was to probe some of the chemical and physico-chemical properties of bovine saliva which may influence rumen digestion and synthesis.

EXPERIMENTAL PROCEDURE

During the course of this investigation, study was made of 77 samples of saliva collected from the unstimulated secretion (except for tactile, visual and auditory stimulation) of 42 Guernsey, Jersey, Holstein and Brown Swiss cattle

of varying ages. Fifty-four samples were collected under neutral paraffin oil to prevent carbon dioxide loss, while 23 samples were taken without oil protection. Blood samples were drawn at the time of saliva collection for ascorbic acid analysis.

The pH of 77 saliva specimens was determined using a Beckman pH meter equipped with a glass electrode. Ascorbic acid was measured in 75 samples according to the method outlined by Mindlin and Butler (24) with modifications (29). The water content of 36 specimens was determined by drying to constant weight. Ascorbic acid analyses were made on corresponding blood plasma samples. The surface tension of 55 samples of saliva was measured by a torsion-bal-

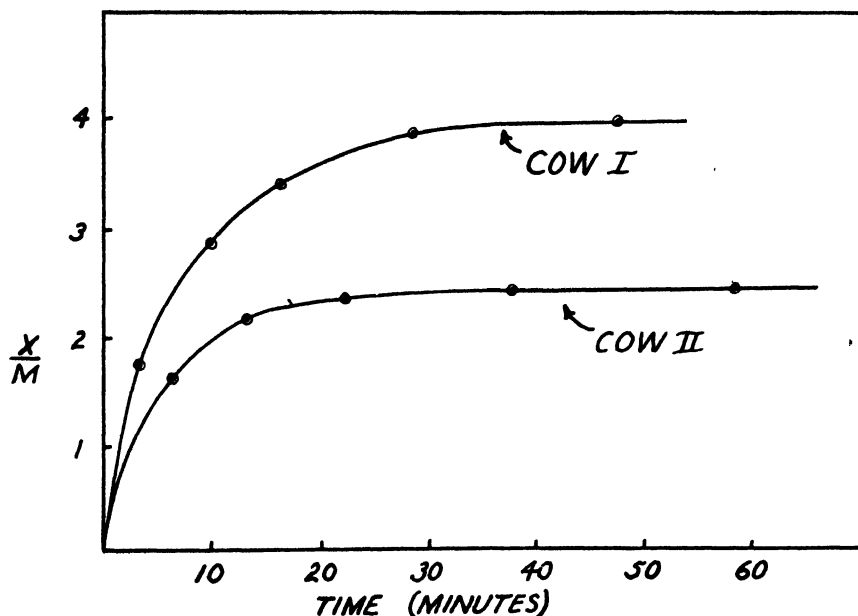


FIG. 1. Adsorption of benzene vapor on the saliva of 2 different cows.

$\frac{X}{M}$ = mg. benzene vapor adsorbed per g. saliva.

ance method in which a Du Nouy tensiometer was employed. The deodorizing capacity of saliva was tested by comparing the odor evolved from various quantities of finely powdered garlic suspended in saliva to that evolved by garlic in distilled water. The possibility of saliva possessing an adsorptive property was investigated by passing benzene at constant temperature (27° C) and pressure (3 bubbles per second) through a U-type adsorption tube containing known quantities of cow's saliva. The amount of benzene taken up by the saliva after exposure for different time intervals was determined by weight. The $\frac{X}{M}$ values (amount of benzene taken up per unit weight of saliva) were plotted against the time exposed (fig. 1).

Similar experiments were conducted in which dry, powdered saliva taken from 26 cows (fig. 2) was employed in place of fresh natural saliva, and in which distilled water was substituted for saliva.

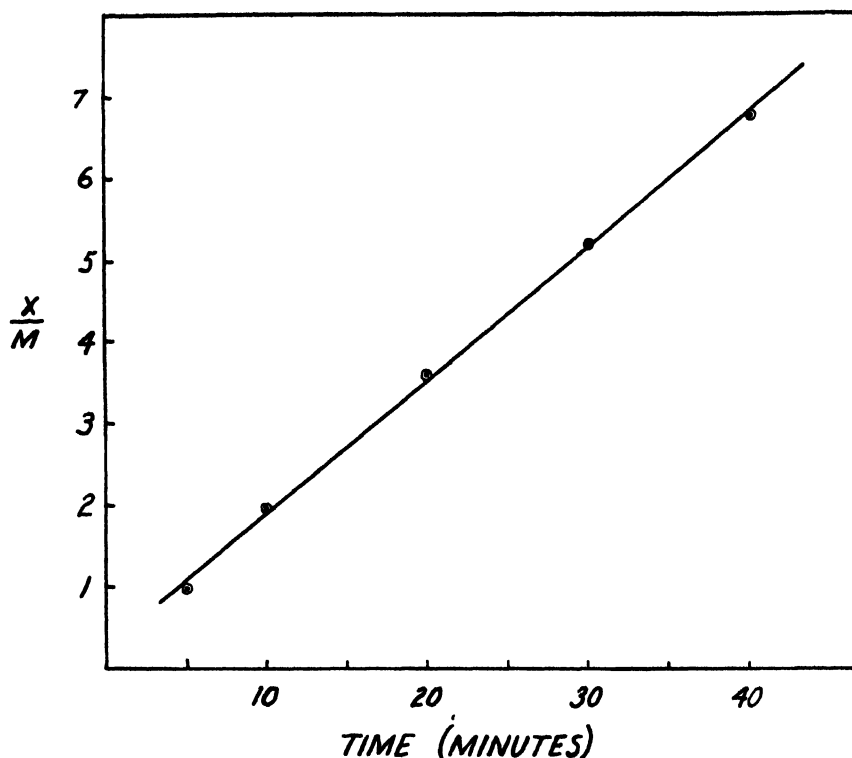


FIG. 2. Absorption of benzene by dried saliva of cows.

$\frac{X}{M}$ = mg. benzene vapor absorbed per g. dried saliva.

RESULTS

The average pH of 23 saliva samples not collected under oil was 8.71, whereas that for 54 samples protected by oil from carbon dioxide loss was 8.53. Some representative values for each are shown in tables 1 and 2. These data suggest that it is imperative to record the conditions under which saliva is collected from animals, i.e. whether or not the specimens are protected from carbon dioxide loss. No great variation was observed in the pH of saliva collected over a 24-hour period, and the time of collection relative to the feeding time had no noticeable effect on the reaction of the saliva.

An analysis of 75 saliva samples revealed an average concentration of 0.15 mg. per cent ascorbic acid, while plasma samples from the corresponding animals at the time of saliva collection had an average ascorbic acid content of 0.47 mg. per cent. Table 2 shows that the ascorbic acid content of saliva collected at intervals

of 6 hours over a 24-hour period was variable and bore no appreciable relationship to the plasma ascorbic acid level. The correlation coefficient between the ascorbic acid level of the plasma and saliva was + 0.015.

The average surface tension of 55 saliva samples was 47.1 dynes per cm. at 29° C. Data obtained from the benzene uptake by fresh saliva which had been exposed for varying time periods derived typical adsorption curves (fig. 1), which indicate that saliva contains adsorptive substances. However, when a similar experiment was conducted on dry, powdered saliva taken from 26 cows, a curve was obtained from the benzene up take which is suggestive of absorption (fig. 2). It seemed, therefore, that the adsorptive property of natural saliva is destroyed by drying.

When water was substituted for saliva in a similar trial, weight losses were noted which were attributed to evaporation.

TABLE 1

Representative data on some chemical and physical properties of cow's saliva and the plasma ascorbic acid level

Animal no.	Plasma		Saliva		
	Ascorbic acid	Ascorbic acid	Water	Surface tension	pH
	(mg. %)	(mg. %)	(%)	(dynes/cm.)	
A5	0.55	0.19	99.10	48.07	8.55
A18	0.42	0.13	98.83	46.05	8.50*
A31	0.72	0.07	99.23	47.97	8.80
A46	0.49	0.06	99.60	45.54	8.62*
A49	0.62	0.16	98.93	46.96	8.56*
B58	0.59	0.13	98.70	47.46	8.63*
C553	0.20	0.02		49.20	8.76
127	0.37	0.21	99.01	45.64	8.93

* Samples collected under neutral paraffin oil.

It was found that 60 mg. of powdered garlic suspended in 100 ml. of distilled water evolved the pungent garlic odor, whereas no characteristic garlic odor was detected by 20 innocent persons from the same amount of the substance suspended in 100 ml. of cow saliva. The elimination of odors by saliva was considered to be an adsorption phenomenon in view of the great surface activity of bovine saliva.

DISCUSSION

The early studies of the role of saliva in digestion concerned the importance of this secretion as a mechanical digestive aid by virtue of its ability to moisten, dissolve and lubricate the dry feeds which constitute the cow's ration. Recent interest in the chemistry and physiology of rumen fermentation and synthesis gave impetus to a study of the constituents and properties of saliva which may influence numbers and kinds of microorganisms.

Greater importance now is being attached to the buffering ability of ruminant saliva than formerly. The large quantity of saliva secreted, its reaction and its activity as a buffer on the alkaline side of a pH of 4 are believed to be largely responsible for the maintenance of a medium which appears to be optimal for micro-

TABLE 2
Blood plasma and saliva ascorbic acid and saliva pH at intervals during a 24 hr. period

Time	Ascorbic acid (mg. %)										Saliva pH ^d				
	Plasma					Saliva									
	A33	A44	A50	419	C553	A33	A44	A50	419	C553	A33	A44	A50	419	C553
A.M. 12:30-1:30	0.59	0.41	0.56	0.68	0.36	0.11	0.18	0.20	0.06	0.03	8.54	8.62	8.48	8.51	8.57
A.M. 6:40-7:30 ^a	0.53	0.22	0.50	0.42	0.20	0.16	0.03	0.13	0.14	0.02	8.53	8.53	8.56	8.60	8.76
P.M. 12:00-12:45 ^b	0.58	0.35	0.42	0.39	0.27	0.13	0.14	0.05	0.11	0.05	8.57	8.45	8.60	8.41	8.48
P.M. 6:10-6:35 ^c	0.55	0.30	0.60	0.69	0.37	0.09	0.03	0.27	0.27	0.03	8.53	8.46	8.40	8.50	8.49

^a Samples collected before morning feeding.

^b Samples collected before afternoon feeding.

^c Samples collected after afternoon feeding.

^d Samples collected under neutral paraffin oil.

bial activity and for the chemical changes occurring in the rumen. The saliva secreted by a mature animal during 24 hours contains approximately 300 to 350 g. of sodium carbonate according to Colin (11), which is theoretically capable of neutralizing 56.6 to 65.0 liters of 0.1 N hydrochloric acid solution.

The alkalinity of dairy cattle saliva offers the possibility that it might absorb some of the carbon dioxide produced in the rumen (22). The increased amount of saliva secreted at the time of eating, regurgitation and rumination may be nature's compensation for the great amount of fermentation during and immediately following eating.

A cow secreting 56 liters of saliva daily excretes approximately 86 mg. of ascorbic acid by this route. Although no explanation has been proposed for the significance of this saliva constituent, the possible use of the substance in saliva could be theorized purely on the basis of its action in other biological systems: (a) the control of hydrolytic enzyme systems, (b) the regulation of oxidation-reduction systems, since it is known to function in H transfers, (c) the detoxification of chemicals such as lead and arsenic by combining with these substances, (d) the initiation of the synthesis of some proteins, since it is known that certain amino acids such as leucine are dehydrated to form ammonia and keto-acids, (e) the inhibition of some organisms which may otherwise be pathogenic to the mouth, esophagus and lower digestive tract, and (f) the encouragement or stimulation of fermentation by certain organisms, since it is known that some of the microorganisms inhabiting the rumen use vitamin C in their metabolism.

To the saliva of the cow has been credited the deodorizing of undesirable barn odors, and hence the elimination of some of the off-flavors of milk without a known explanation for the deodorizing process. A comparison of the odor of garlic suspended in water and saliva demonstrated that no characteristic odor was evolved from the saliva while a pungent odor was readily detected from garlic in water. Since the deodorizing phenomenon generally is regarded as one of adsorption, as shown in water-odor purification studies by Baylis (4, 5) and Spalding (34), a phase of this investigation was given to the study of the surface activity of saliva. Natural saliva demonstrated typical adsorption tendencies, whereas dried saliva gave results characteristic of absorption. It is known that a considerable portion of the solid matter in saliva is protein; Bramkamp (8) found 250 mg. per cent protein, and Inouye *et al.* (16) reported that saliva contains 260 mg. per cent mucinate. Zolnikova (41) reported that 86 to 89 per cent of the total nitrogen of saliva is mucin nitrogen. According to Bucher (9), the mucoid proteins of gastric mucus are extremely small particles which are not visible using the ultramicroscope, but are amorphous-like in nature and possess colloidal properties. Since Levene (20) has shown that the protein complex from one mucin to another differs chiefly in the nature of their amino-hexoses, it appears that the mucoid proteins of saliva also are of small molecular size which would facilitate adsorption by virtue of their great surface area. Mahlo (21) stated that the hydrochloric acid of gastric juice is adsorbed partly by mucin. Investigations by Bradley (7) and Babkin (3) demonstrated the capability of

mucin as an adsorbent. From an analysis of the foregoing studies it seemed logical to attribute the adsorption properties of cow's saliva to mucoid proteins.

Although the uptake of benzene by saliva was in small quantities, the same test applied to water showed a loss of weight even when exposed only one minute and progressive decreases thereafter which seemed to be attributable to evaporation. The amount of benzene taken up by the saliva could not be accounted for by solubility, because the quantity of benzene which can be dissolved in the amount of water contained in the saliva (0.70 mg. of benzene per g. of saliva at 22° C.) is a very small portion of the amount actually adsorbed during any time interval.

The low surface tension (47.10 dynes per cm.) of dairy cattle saliva is an amazing fact when one perpend its high water content (99.12 per cent) or compares it to a well known surface tension depressant such as a 0.1 per cent sodium oleate solution which has a surface tension of 42.2 dynes per cm. and to pure water with a surface tension of 71.35 dynes per cm. at 30° C. This property, indicating wetting ability, offers a plausible account for the efficiency of saliva in moistening feed materials.

McCulloch (23) stated that the surface tension of bacterial cultural media usually is 50 to 60 dynes. Larson *et al.* (17, 18) showed that the amount of growth of certain bacteria depends to a great extent upon the surface tension of the media. Albus and Holm (1) reported that *Lactobacillus acidophilus* grew well at a surface tension of 36 dynes, whereas *Lactobacillus bulgaricus* could not initiate growth in a medium whose surface tension was reduced to 40 dynes per cm. These workers (1) believed that a low surface tension is responsible for the failure to obtain a permanent implantation of *L. bulgaricus* in the normal human intestinal tract, whereas *L. acidophilus* implantations were successful. Probably the low surface tension of saliva is optimal for the bacteria types indigenous to the rumen and may be selective to the extent of limiting the kind of organism present.

In view of the surface energy exerted by the large quantity of saliva secreted by the cow, the efficiency of chemical reactions and bacterial functions occurring in the rumen by virtue of wetting, solution and mixing, in addition to maintaining the proper rumen reaction, seem responsible to saliva activity.

SUMMARY

An investigation was conducted on 77 samples of saliva from dairy cattle to ascertain some of this secretion's chemical and physico-chemical properties. The average water content of 36 specimens was 99.12 per cent.

The average pH of 54 samples protected by neutral paraffin oil from carbon dioxide loss was 8.53, whereas the average pH for the unprotected samples was 8.71. No appreciable variation was noted in the pH of saliva collected at 6-hour intervals over a 24-hour period, and the time of collection relative to the feeding time had no apparent effect on the reaction of the saliva.

An analysis of 75 saliva samples showed an average ascorbic acid concentra-

tion of 0.15 mg. per cent, while the average plasma ascorbic acid level of the same animals was 0.47 mg. per cent.

The deodorizing and surface active properties of cow's saliva were demonstrated. The surface tension of 55 samples was 47.10 dynes per cm. at 29° C., while typical adsorption curves were derived from the uptake of benzene by saliva. Since dried cow's saliva took up benzene in a manner characteristic of adsorption, it was believed that the adsorbent materials of saliva were altered in some way by drying, resulting in the loss of the adsorbing property.

REFERENCES

- (1) ALBUS, W. R., AND HOLM, G. E. Surface Tension as a Factor in the Growth of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. Abstracts Bact., 9: 142. 1925.
- (2) BABICHEV, G. A., PERSTNOV, N. S., AND KULESCO, I. I. The Work of the Digestive glands in Ruminant Animals. II. Secretory Nerve Apparatus of Gland Parotis in Goats. Russ. Physiol. J., 13: 636. 1930. (Cited by Babkin (3).)
- (3) BABKIN, B. P. Secretory Mechanism of the Digestive Glands. Paul B. Hoeber, Inc., New York. 1944.
- (4) BAYLIS, J. R. Activated Carbons and Their Use in Removing Tastes and Odors from Water. J. Am. Water Works Assoc., 21: 787-814. 1929.
- (5) BAYLIN, J. R. Further Observations on the Use of Activated Carbon in Removing Objectionable Tastes and Odors from Water. J. Am. Water Works Assoc., 22: 1438-1461. 1930.
- (6) BORISSOVSKY, V., AND WWEDENSKY, N. Oberflächenaktivität des menschlichen Speichels. Biochem. Z., 219: 79-86. 1930.
- (7) BRADLEY, H. C. Inhibition of Pepsin by Mucin. (Proc. Am. Soc. Biol. Chem.) J. Biol. Chem., 100: xx. 1933.
- (8) BRANKAMP, R. G. The Protein Content of Human Parotid Saliva. J. Biol. Chem., 114: 369-371. 1936.
- (9) BUCHNER, R. Das Wesen der Schutzwirkung des Magenschleims. Deut. Z. Chir., 236: 515-559. 1932. (Cited by Babkin (3).)
- (10) CHRZASZCZ, T., AND SCHECTŁÓWNA, Z. Die Amylase des Speichels der Rinder und Pferde. Biochem. Z., 219: 30-50. 1930.
- (11) COLIN, G. C. Traite de Physiologie comparee des Animaux, Vol. 1, 3rd Ed. J. B. Bailliere et Fils, Paris. 1886.
- (12) DUKES, H. H. The Physiology of Domestic Animals, 5th Ed. Comstock Publ. Co., Ithaca, New York. 1943.
- (13) FOMIN, D. A. Secretion from the Salivary Glands of Calves. J. Physiol. U.S.S.R., 30: 524-527. 1941.
- (14) HELD, A. J., HELD, H. R., AND GUILLERMAN, P. Reducing Powers of Saliva (translated title). Schweiz. Monatschr. Zahnkeilk., 48: 280-287. 1928.
- (15) HOEFFNER, H. Über Strukturviskosität und Schaumvermögen des menschlichen Speichels. Kolloid-Z., 105: 216-223. 1943.
- (16) INOUE, J. M., FORER, S., REISCHE, M. G., AND MILLER, F. G., JR. The Mucinate Content of Saliva. Proc. Soc. Exptl. Biol. Med., 25: 153-155. 1927.
- (17) LARSON, L. W., AND LARSON, W. P. Factors Governing the Fat Content of Bacteria and the Influence of Fat Pellicle Formation. J. Infectious Diseases, 31: 407-415. 1922.
- (18) LARSON, W. P., CANTWELL, W. F., AND HARTZELL, T. B. The Influence of the Surface Tension of the Culture Medium on the Growth of Bacteria. J. Infectious Diseases, 25: 41-46. 1919.
- (19) LASSAIGNE, J. J. de Chemie medicale, 8 (3rd series): 397. 1852. (Cited by Colin (11).)

- (20) LEVENE, P. A. Hexosamines and Mucoproteins. Monographs on Biochemistry. Longmans, Green and Co., London. 1925.
- (21) MAHLO, A. Der Magenschleim. Ferd. Enke, Stuttgart. 1938. (Cited by Babkin (3).)
- (22) MARKOFF, J. Untersuchungen über die Gärungsprozesse bei der Verdauung der Wiederkäuer. Biochem. Z., **34**: 211-232. 1911.
- (23) McCULLOCH, E. C. Disinfection and Sterilization. Lea and Febiger, Philadelphia. 1945.
- (24) MINDLIN, R. L., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma; a Macromethod and Micromethod. J. Biol. Chem., **122**: 673-686. 1938.
- (25) PAVLOV, I. P. The Work of the Digestive Glands. (Translation by W. H. Thompson.) 2nd English Ed., Charles Griffin and Co., Limited, London. 1910.
- (26) PINCUS, P. Saliva: An Account of a Property Previously Undescribed. Brit. Dental J., **68**: 96-97. 1940.
- (27) PINCUS, P. The Reducing Properties of Saliva. Brit. Dental J., **72**: 181-188. 1942.
- (28) POPOV, N. A. Physiology of the Sheep. Moscow. 1932. (Cited by Babkin (3).)
- (29) REID, J. T., AND SYKES, J. F. The Influence of Ascorbic Acid on the Activity of Gonadotropic Hormones in Guinea Pigs. J. Nutrition, **30**: 477-483. 1945.
- (30) SCHEUNERT, A., AND TRAUTMANN, A. Studium der Speichelsekretion. II. Über die Sekretion der Parotis und Mandibularis des Schafes. Pflügers Arch. ges. Physiol., **192**: 33-69. 1921.
- (31) SCHWARTE, L. H. Unpublished data. 1939. (Cited by Dukes (12).)
- (32) SCHWARZ, C., AND HERMAN, B. Beiträge zur Physiologie der Verdauung. Die H-Ionenkonzentration im Speichel einiger Haustiere. Pflügers Arch. ges. Physiol., **202**: 475-477. 1924.
- (33) SHARMA, G. K. Secretion and Composition of Parotid Saliva in Buffaloes. Indian J. Vet. Sci., **6**: 266-269. 1936.
- (34) SPALDING, G. R. Activated Charcoal as a Deodorant in Water Purification. J. Am. Water Works Assoc., **22**: 646-648. 1930.
- (35) STUTEVILLE, O. H. Presence of Vitamin C in Saliva. Proc. Exptl. Biol. Med., **32**: 1454-1455. 1935.
- (36) SUZUKI, R. Biochemical Study of the Salivary Glands. I. The Amount of Ascorbic Acid in the Salivary Glands under Various Conditions. Sei-i kai Med. J., **57**: 4-5. 1938.
- (37) SUZUKI, R. Biochemical Study of the Salivary Glands. II. The Glutathione Content of the Salivary Glands under Various Conditions. Sei-i kai Med. J., **58**: 5-7. 1939.
- (38) WISE, G. H., MILLER, P. G., AND ANDERSON, G. W. Changes Observed in Milk "Sham-fed" to Dairy Calves. J. Dairy Sci., **23**: 997-1011. 1940.
- (39) WWEDENSKY, N. Oberflächenenergie einiger physiologischer Flüssigkeiten. Biochem. Z., **189**: 270-278. 1927.
- (40) ZIMMET, D., AND DUBOIS-FERRIERE, H. I. Variations with Age in the Reducing Power (Vitamin C) of Human Saliva. II. Vitamin C in the Saliva of Children with Various Infectious Diseases. (Translated title) Comp. Rend. Soc. Biol., **124**: 246-248. 1937.
- (41) ZOLNIKOVA, N. K. Alterations of Different Nitrogenous Fractions in the Mixed Saliva from the Submaxillary and Sublingual Glands During Exhaustion and the Period of Restitution. (Translated title.) Sochenov. Physiol. J. U.S.S.R., **30**: 642-646. 1941.

THE INFLUENCE OF TOCOPHEROLS AND COD-LIVER OIL ON MILK AND FAT PRODUCTION

FRANK WHITING, J. K. LOOSLI, VLADIMIR N. KRUKOVSKY, AND K. L. TURK

*Departments of Animal Husbandry and Dairy Industry,
Cornell University, Ithaca, N. Y.*

Numerous studies have shown that feeding cod-liver oil to dairy cows and goats markedly reduces the fat percentage of the milk (7, 8, 21, 24). Little is known about the specific cause of this depression of the fat test, but it has been shown that hydrogenated cod-liver oil is no longer active (8, 22). Moore *et al.* (23) observed that giving cod-liver oil to cows in 12 feedings each day caused no such marked depression of test in a 6-day feeding period as when the oil was fed in a single daily dose. Certain other fats and oils have been shown to cause an increase, at least temporary, in the fat content of the milk of cows and goats (16).

Recently, Harris *et al.* (10) have reported that the percentage of fat in the milk of dairy cows was increased approximately 27 per cent by feeding 1 g. of mixed natural tocopherols daily over a 20-month period.

The foregoing reports and the established antagonistic action between cod-liver oil and alpha-tocopherol in nutritional muscle dystrophy (19) led the authors to wonder if a similar interrelationship might exist in the secretion of milk fat by cows. Accordingly, an experiment with cows was designed to study further the effect of tocopherols upon milk production and particularly to determine whether tocopherols would inhibit the depressing influence of cod-liver oil on the fat percentage.

EXPERIMENTAL PROCEDURE

Sixteen cows of the Holstein, Brown Swiss and Guernsey breeds were used in this study during the winter of 1947-48. The cows were divided into four equal groups according to age and breed. The first group consisted of mature Brown Swiss cows; the second group, 2-year old Brown Swiss cows; the third group, 2-year old Holstein cows and the fourth group, mature Guernsey cows. Four replicates of 4×4 Latin squares were used. Control ration A consisted of medium quality mixed grass and legume hay, corn silage and a commercial 16 per cent protein dairy concentrate mixture. Ration B was the control ration plus 1 g. of mixed natural tocopherol.¹ Ration C was ration A plus 5 oz. veterinary grade cod-liver oil.² Ration D consisted of ration A plus 5 oz. veterinary grade cod-liver oil and 1 g. of mixed tocopherols.

Received for publication August 26, 1948.

¹ A stabilized tocopherol concentrate "Myvadry" purchased from Distillation Products, Inc., Rochester, N. Y.

² Contained 2000 units of vitamin A and 100 units of vitamin D per g. Purchased from Marine Products Co., Boston, Mass.

One lb. of grain was fed for each 3 lb. of 4 per cent fat-corrected milk produced during the preliminary period. During the experiment, the grain allowance was reduced in accordance with the average decline in production of all cows as recommended by Lucas (17). A constant amount of silage, approximately 25 lb. daily per cow, was fed throughout the experiment. Hay was fed ad libitum.

The tocopherol supplement was placed on top of each cow's morning feeding of concentrate. Cod-liver oil was mixed with a small amount of molasses and poured on the silage at the evening feeding. The cod-liver oil and tocopherols were fed at separate feedings, because Mackenzie *et al.* (19) had shown that the muscular-dystrophic preventative action of tocopherol was rendered ineffective when it was administered with cod-liver oil.

Each cow was started on experiment approximately 60 days after parturition, following a preliminary period of 3 weeks, thus all cows were in the same stage of lactation. Each period was of 4 weeks duration and was followed by 1 week on the control ration before starting the next period.

Milk was weighed and sampled at each milking. These aliquots were composited for seven milkings, and butterfat determinations by the Babcock test were made on the composited aliquots twice weekly. At the end of each 4-week period, samples of blood were obtained for tocopherol, vitamin A and carotenoid analyses. A 2 quart sample of milk was taken from the morning milking at the end of each 4-week period for studies on tocopherol, vitamin A and carotene content and keeping qualities.

The method of Koehn (14) was used to measure the vitamin A and carotenoid content of milk fat, while that of Kimble (13) was used to determine the vitamin A and carotenoid content of blood plasma. The procedure of Quaife (25) was used to determine the tocopherol content of milk fat and that of Quaife and Harris (27), with the microhydrogenation apparatus of Quaife and Biehler (26), to determine the tocopherol content of the blood plasma.

In an additional study, six Holstein cows were used in a single-reversal experiment during the summer of 1947 to study the influence on milk and fat production of feeding 1 g. daily of mixed natural tocopherols to cows on pasture. In this experiment, the cows were divided into two groups of three cows each; the two groups were equalized in daily milk production, state of lactation and fat test. The supplemental periods were of 5 weeks duration. The cows were on good pasture and received a concentrate allowance in accordance with milk production (1 lb. of concentrate per 4 lb. of milk produced). The method of feeding the supplement and of handling the milk was essentially the same as described above.

RESULTS

The average daily pounds of milk produced, butterfat tests, pounds of fat produced and the pounds of 4 per cent fat-corrected milk (6) are presented in table 1. Only the milk and fat yields obtained during the last 2 weeks of each period were used in calculating these averages, since the maximum fat depress-

ing effect of cod-liver oil was not evident until the second week; also Harris *et al.* (10) reported that the stimulating effect of tocopherol on fat percentage was not evident until approximately 10 days after starting to feed the supplement. In the experiment with the cows on pasture, milk and butterfat yields during the last 3 weeks were used to calculate averages, since the periods in this experiment were of 5 weeks duration.

Feeding 1 g. of mixed tocopherols daily over a 4-week feeding period slightly increased the fat test, the average daily lb. of milk and fat produced, and the average daily pounds of 4 per cent fat-corrected milk (table 1). However, none of these increases was statistically significant. These results do not confirm the findings of Harris *et al.* (10), but it should be emphasized that the length of the feeding period in this experiment was only 4 weeks (5 weeks on pasture), whereas Harris *et al.* used a 20-month experimental period.

TABLE 1

The influence of tocopherols and cod-liver oil upon the average butterfat percentage, total milk yield and 4% fat corrected milk

Supplement	Butterfat	Average daily production		
		Milk	Fat	4% F.C.M.
	(%)	(lb.)	(lb.)	(lb.)
Winter, 1947-48				
None	4.24	30.3	1.28	31.4
Tocopherols	4.30	30.7	1.32	32.1
Cod liver oil	3.63	32.8	1.19	31.0
Tocopherols and cod-liver oil	3.63	32.0	1.16	30.3
Summer, 1947* (pasture)				
None	3.55	35.7	1.27	33.3
Tocopherols	3.58	35.2	1.26	33.0

* Holstein cows only.

Our results are in agreement with those of Gullickson *et al.* (9), who observed no increase in the fat yield or fat percentage from feeding tocopherols.

Feeding 5 oz. of cod-liver oil alone or with tocopherol caused a 14 per cent decrease in the average fat test, a slight decrease in average daily pounds of 4 per cent fat-corrected milk, but a 6 per cent increase in average daily pounds of milk produced. The decrease in fat test and the increase in milk yield were both highly significant (1 per cent level of probability), using the method of Cochran *et al.* (3) to analyze the data. The fat depressing effect of cod-liver oil observed in this experiment agrees with the findings of others, but no one has reported an increase in milk yield due to feeding cod-liver oil. Petersen (24) and McCay and Maynard (21) reported that milk production was not affected by cod-liver oil feeding. It has been shown previously that feeding extra vitamin A does not increase milk production (12, 18). No explanation is offered for the increase in milk production found in these investigations from feeding cod-liver oil.

Feeding tocopherols did not prevent the drop in fat test due to feeding cod-liver oil. Apparently the supplemental tocopherol was not destroyed by the cod-liver oil, since the tocopherol content of the butterfat and the blood of the cows when receiving tocopherol and cod-liver oil were essentially the same as when they received tocopherol alone (table 2). Feeding cod-liver oil alone, however, did lower the tocopherol content of the butterfat and milk, indicating that cod-liver oil destroys tocopherols in the dairy cow in a manner similar to that observed in other animals.

Cod-liver oil depressed the carotene content of the blood and milk fat and increased the vitamin A content. A similar phenomenon has been observed by other investigators when high levels of vitamin A have been fed (2, 5, 10, 29). However, when both tocopherols and cod-liver oil were fed, the carotene-depressing effect of the cod-liver oil was not so pronounced. Harris *et al.* (10)

TABLE 2

The tocopherol, vitamin A and carotenoid content of milk fat and blood plasma as affected by tocopherol and cod-liver oil supplements

Supplement	Content in milk fat of:			Content in blood plasma of:		
	Toco- pherols	Vitamin A	Caroten- oids	Toco- pherols	Vitamin A	Caroten- oids
		($\gamma/100$ g.)			($\gamma/100$ ml.)	
None	2990	559	440	582	27.8	528
Tocopherols	3569	544	390	735	28.8	463
Cod-liver oil	2529	1301	326	427	31.5	368
Tocopherols and cod-liver oil	3590	1329	349	696	34.3	438
None*	3190			685	22.2	1054
Tocopherols*	3640			771	23.9	1106

* Holstein cows only.

noted that feeding tocopherols with a vitamin A concentrate largely prevented the carotene depressing effect of the vitamin A supplement.

Of special interest is the fact that tocopherol feeding improved the ability of milk to resist the development of off-flavors as described in detail by Krukovsky *et al.* (15).

SUMMARY

Feeding 1 g. of natural mixed tocopherols daily to dairy cows during both winter and pasture feeding for periods of 4 to 5 weeks had no significant influence on milk or fat production. However, feeding 5 oz. of cod-liver oil daily to cows during winter feeding decreased the butterfat percentage approximately 14 per cent, but increased the total milk production 6 per cent. Tocopherol supplementation did not counteract the butterfat depressing effect of cod-liver oil.

The tocopherol content of the milk fat and blood plasma was increased by feeding tocopherols, but was decreased by feeding cod-liver oil. Cod-liver oil, had a depressing effect on the carotene content of the milk fat and blood plasma,

but this was counteracted somewhat by feeding tocopherols with the cod-liver oil. The vitamin A content of the milk fat and blood plasma was increased by feeding cod-liver oil.

REFERENCES

- (1) ALLEN, N. N. The Fat Percentage of Milk as Affected by Feeding Milk to Dairy Cows. *J. Dairy Sci.*, **15**: 132-141. 1932.
- (2) BLAXTER, K. L., KON, S. K., AND THOMPSON, S. Y. The Effect of Feeding Shark Liver Oil to Cows on the Yield and Composition, and on the Vitamin A and Carotene Content of the Milk. *J. Dairy Research* **14**: 225-230. 1946.
- (3) COCHRAN, W. G., AUTREY, K. M., AND CANNON, C. Y. A Double Change-over Design for Dairy Cattle Feeding Experiments. *J. Dairy Sci.*, **24**: 937-951. 1941.
- (4) EPSTEIN, S. H., AND MORGULIS, S. Factors Affecting the Onset and Cure of Nutritional Muscular Dystrophy. *J. Nutrition*, **23**: 473-482. 1942.
- (5) FOUNTAINE, F. C., AND BOLIN, D. W. The Milk and Butter Fat Production Responses to Shark Liver Oil in the Ration. *J. Dairy Sci.*, **27**: 155-158. 1944.
- (6) GAINES, W. L. The Energy Basis of Measuring Milk Yields in Dairy Cows. *Ill. Agr. Expt. Sta. Bull.* 308. 1928.
- (7) GOLDING, J., SOAMES, K. M., AND ZELVA, S. S. The Influence of Cows Diet on the Fat-soluble Vitamins of Winter Milk. *Biochem. J.*, **20**: 1306-1319. 1926.
- (8) GRAHAM, W. R., AND CUPPS, P. T. The Action of Herring Oil Before and After Hydrogenation on the Yield and Fat Percentage of the Milk of the Goat. *J. Dairy Sci.*, **21**: 45-48. 1938.
- (9) GULLICKSON, T. W., FITCH, J. B., AND GILMORE, L. O. Effect of Feeding Tocopherols to Dairy Cows on the Quantity and the Fat Content of Milk Produced. *J. Dairy Sci.*, **31**: 557-560. 1948.
- (10) HARRIS, P. L., SWANSON, W. J., AND HICKMAN, K. C. D. Covitamin Studies VI. Effect of Tocopherol Supplementation on the Output of Vitamin A, Carotene, and Fat by Dairy Cows. *J. Nutrition*, **33**: 411-427. 1947.
- (11) HOVE, E. L., AND HARRIS, P. L. Relative Activity of the Tocopherols in Curing Muscular Dystrophy in Rabbits. *J. Nutrition*, **33**: 95-106. 1947.
- (12) JENSEN, C., BOYER, P. D., PHILLIPS, P. H., RUPEL, I. W., AND LUNDQUIST, N. S. The Effect of Shark Liver Oil on Milk and Butterfat Production. *J. Dairy Sci.*, **25**: 931-937. 1942.
- (13) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, **24**: 1055-1065. 1939.
- (14) KOHN, C. J. Vitamin A Activity of Milk as Related to Pasture and Feeding Practices in Alabama. *J. Dairy Sci.*, **26**: 673-681. 1943.
- (15) KRUKOVSKY, VLADIMIR N., LOOSLI, J. K., AND WHITING, FRANK. The Influence of Tocopherols and Cod Liver Oil on the Stability of Milk. *J. Dairy Sci.*, **32**: 133-138. 1949.
- (16) LOOSLI, J. K., VOLZ, FRANCES, AND LUCAS, H. L. Cornell Univ. Unpublished Data. 1948.
- (17) LUCAS, H. L. A Method of Equalized Feeding for Studies with Dairy Cows. *J. Dairy Sci.*, **26**: 1011-1022. 1943.
- (18) LUCAS, H. L., LOOSLI, J. K., AND MAYNARD, L. A. A Study of the Effect of Dietary Fat and Fat-soluble Vitamins upon Milk and Fat Secretion. *Cornell Memoir* 251. 1943.
- (19) MACKENZIE, C. G., MACKENZIE, J. B., AND MCCOLLUM, E. V. Uncomplicated Vitamin E Deficiency in the Rabbit and its Relation to the Toxicity of Cod Liver Oil. *J. Nutrition*, **21**: 225-234. 1941.
- (20) MATTILL, H. A., AND GOLUMBIC, C. Vitamin E, Cod Liver Oil, and Muscular Dystrophy. *J. Nutrition*, **23**: 625-631. 1942.

Sisson (88) observed that from 10 to 15 per cent of lactose added to a cow's-milk diet produced diarrhea in some puppies but not in others. Younger puppies developed diarrhea sooner than did older ones. The diarrheal condition was alleviated quickly by omitting the lactose from the milk mixture. Gross (36) was not able to feed diets containing more than 15 per cent of lactose without diarrhea appearing, and even the 15 per cent level produced diarrhea in about half of the dogs used. Dragstedt and Peacock (23) observed that the feces of 2 to 3-months-old puppies became liquid in from 4 to 6 days, when a diet of white bread and whole milk was fed with 60 g. of lactose per day. Adult dogs fed a diet of 500 g. of rice, 100 g. of beef heart and 50 g. of lactose did not have liquid feces, but when fed white bread and skim milk *ad libitum*, plus 50 to 125 g. of lactose, most of the adult dogs developed liquid feces after 1 week. This difference in the fluidity of the feces, depending upon the constitution of the diet to which the lactose was added, perhaps may be due to the other constituents of the diet either having some direct effect on fecal consistency or influencing the lactose effect. Diarrhea was noted by French and Cowgill (32) when diets containing 20 per cent of lactose were fed to dogs.

That the rat apparently is better able to utilize lactose than the dog was the conclusion reached by Koehler and Allen (49). They did not observe diarrhea when 35 per cent of lactose was fed to young, full-grown rats. But others have observed diarrhea in adult rats fed diets containing 67 per cent of lactose (85) and 87.5 per cent of lactose (35).

Outhouse *et al.* (67) did not observe diarrhea in their young rats which were fed 25 per cent of lactose in the ration. Whittier *et al.* (101) observed diarrhea in rats at 45 and 63.5 per cent levels of lactose feeding. Mitchell and Dodge (62) reported that diarrhea was exhibited by young rats placed on a 70 per cent lactose ration. Mitchell (60) confirmed this observation for all rat strains used by her, and noted that 35 per cent of *galactose* in the ration did *not* produce the diarrheal effect. Mitchell *et al.* (56) also showed that rations containing 60 to 70 per cent lactose produced diarrhea and slow growth.

Mitchell *et al.* (61) observed diarrhea in young rats placed on a 50 per cent lactose diet. This diarrhea varied in severity with the individual rat. Coryell and Christman (17) stated that, due to the laxative action, lactose doses for rats cannot be increased much beyond 2 g. per kg. of body weight. This figure represents a considerably lower level of lactose intake for producing laxation than has been reported by any other investigator, but it should be noted that Coryell and Christman worked with rats fasted for 24 hours. Diarrhea was observed by Boutwell *et al.* (10) for the first 6 to 12 days of feeding a ration containing 48 per cent lactose and 28 per cent corn oil to weanling rats.

Diarrhea followed by death of rats fed large amounts of lactose in the ration was reported by Evans and Burr (28). Others who have reported a similar occurrence are Fisher (30), Morgan *et al.* (63), and Skeggs and Wright (89), when they fed rations containing 77, 65, and 61.8 per cent of lactose, respectively. Ershoff and Deuel (26) reported that weanling rats fed a ration containing 73.2

per cent lactose or β -lactose developed a severe diarrheal condition and died. The length of time these rats survived depended upon the severity of the diarrhea. The β -lactose rats died sooner than did the lactose rats, having suffered more severe diarrhea in general than had the lactose rats. Attempts to control the diarrhea by including 5 or 10 per cent of citrus pectin or 5 per cent of apple powder in the diet were not successful.

Scott and Verney (86) noted severe diarrhea in rats fed a 62 per cent lactose diet. They found that when rats were allowed to choose dietary components, lactose was avoided, while other carbohydrates were accepted to various degrees. Richter (78) found that rats offered lactose as the sole food ate very small quantities. The rats survived only 7 days on the average, as compared with 4 days for a comparable group given only water. Another group fed only galactose lived only 7 days. When Ershoff (25) fed lactose to rats as the sole food, all the rats had diarrhea, and they died after the same length of time (5 to 7 days) as if they had been fasted. Other rats fed dextrose, sucrose or butter fat as the sole food lived 34 days on the average. That the diarrhea and early deaths were not due to galactose was shown by feeding a ration composed of 50 per cent dextrose and 50 per cent galactose, in which case no diarrhea was observed and the rats lived about as long as the rats fed dextrose, sucrose or butter fat alone. But feed intake was increased so that the rats obtained their necessary calories from the dextrose.

The most complete determination of the lowest level of lactose which will produce diarrhea in weanling rats (Sprague-Dawley strain) was made by Riggs and Beaty (79). They did not observe diarrhea at lactose feeding levels of 5, 10 or 15 per cent, but they did observe diarrhea beginning at the 20 per cent level and increasing in severity and persistence as the level was raised to 30, 40 and 50 per cent. These investigators also showed that the diarrhea was due to the lactose linkage by feeding equivalent levels of the hydrolysis products, glucose and galactose, without producing any diarrhea.

Few reports are available on the amounts of lactose which will produce diarrhea in man, although considerable use was made of the sugar for complementing the action of *Lactobacillus acidophilus* milk in treating constipation. Since it is difficult to tell how much of the action might be due to the lactose and how much to the effects of the bacteria, these reports will be discussed together in a future section, rather than receive consideration under the present heading. In one of the instances in which lactose was fed alone, (that is, without *L. acidophilus* milk) diarrhea was observed by Robinson (80) when 200 to 250 g. of lactose were ingested by human subjects. Koehler *et al.* (50) observed flatuency and diarrhea in only one case, when from 80 to 120 g. of lactose were ingested. Rettger and Cheplin (76, p. 110) found that 300 g. of lactose per day tended to produce diarrhea in man. Kopeloff and Cohen (53) observed that in 6 of 15 subjects the first ingestion of a 100-g. dose of lactose caused diarrhea. Barker (4) recommended adding from 50 g. to 1 lb. of lactose to the diet of typhoid fever patients, as long as there was no diarrhea. He stated that one should remember that

patients given the high-calorie diet he recommended usually have from 2 to 4 stools a day. Traube (96) reported that a light laxative action was produced by only 9 to 15 g. of lactose taken in the morning on an empty stomach. Albertoni (1) stated that 20 g. of lactose were required for action in most cases.

Rojas *et al.* (83) have reported the occurrence of diarrhea in two dairy calves fed separated milk with extra lactose added. The amount of lactose added brought the total lactose content to twice that of separated milk; that is, lactose intake was about 640 g. per day for the Holstein calf and about 500 g. per day for the Jersey calf. The scouring began within a few hours after beginning the feeding of the lactose-enriched separated milk, and decreased when the extra lactose was omitted.

Species differences in susceptibility to lactose diarrhea are indicated in the above accounts of amounts of lactose which produce diarrhea. However, due to the lack of information on actual amounts of the sugar ingested per unit weight, direct comparisons are almost impossible. But in general, it seems that rats are less susceptible to lactose diarrhea than are dogs, while the position of the human in this respect remains obscure.

Age differences also are indicated. Young animals probably are more susceptible to the diarrhea, as shown by Sisson (88) and Ershoff and Deuel (26). Strains of rats also differ in susceptibility, according to Mitchell (60) and Ershoff and Deuel (26). Individual differences have been reported by Gross (36), Sisson (88), and Riggs and Beaty (79).

THE EFFECTS OF LACTOSE ON INTESTINAL MOTILITY IN INFANTS

The importance of lactose in the diet of the human infant is well-known. Hurst (44, pp. 422-424) mentioned that fat and lactose were the two substances in breast milk which stimulated intestinal movements in infants. He called attention to the fact that since undiluted cow's milk has approximately the same per cent of fat, two-thirds as much lactose and more than twice as much protein as human milk, cow's milk usually is diluted for human infants. This dilution makes the protein percentage more nearly comparable to that of breast milk, but at the same time it reduces the percentage of stimulating lactose further below the normal breast milk level. Hurst further commented that the cure of constipation in the non-breast-fed infant often is accomplished immediately by adding 1 teaspoon of lactose to each 3-4 oz. of undiluted cow's milk. For constipation of the breast-fed baby, he recommended 0.5 to 1 teaspoonful of lactose in a little warm water before each nursing.

Talbot and Hill (94) noted that more than 9 to 14 per cent of lactose in the formula resulted in diarrhea in a 5-months-old infant. More than this amount decreased the absorption and perhaps the retention of nitrogen. Porter and Dunn (74) observed that the largest amount of lactose which could be taken by infants without intolerance (meaning without diarrhea, gas and vomiting) varied from 9 to 40 g. per kg. per day, from 2 to 4.75 g. per kg. at a feeding or from 5

to 15.5 per cent of the diet. Paffrath and Siering-Kaulla (69) observed diarrhea and impaired digestion in all trials when 15 per cent lactose solutions were given to children.

Gerstley *et al.* (34) observed that up to 17 per cent lactose added to breast milk produced little change in the fresh weight of infant stools; however, up to 12 per cent added to cow's milk increased the fresh weight and frequency of the stools, but without producing any diarrhea. In a later paper, Gerstley (33) mentioned that a temporary upset in digestion was accompanied by diarrhea for 1 day when an infant was changed to a formula of cow's milk plus 12 per cent lactose. Gerstley's work was begun with the idea that acids produced in the intestine by fermentation of lactose caused the diarrhea observed in some infants. The theory was that lactose fermented more readily when given in cow's milk than when given in breast milk. However, actual results showed that up to 12 per cent of lactose added to cow's milk actually improved the well-being of the infant and did not cause any diarrhea.

Barenberg and Abramson (3) observed somewhat heavier feces (on the fresh weight basis, presumably, although not stated) but no diarrhea, when 12–15 per cent of lactose (15–17 g. per kg. of body weight) was fed to babies 3 to 15 months old.

Skole (91) mentioned that constipation was notably absent in 108 babies (average age 14 weeks at the start) fed evaporated milk and 0.1 oz. of β -lactose per lb. of body weight. Diarrhea sometimes was precipitated when an infant first was started on the diet, but this was controlled readily by reducing the amount of lactose or by omitting it altogether for 2–3 days and then gradually adding it back.

Dennett and Craig (20) observed more diarrhea among breast-fed infants than among infants fed modified fresh cow's milk, evaporated or powdered milk, and more diarrhea on fresh cow's milk than on evaporated or powdered milk. It was not stated whether the powdered milk was dry whole milk or nonfat dry milk solids. The babies were about 2 months old at the start of the experiment.

Jeans and Marriot (46, p. 63) have called attention to the fact that giving lactose solutions of a 10 per cent or greater concentration may result in an increase in the number of stools. Also (46, p. 70) lactose or sucrose solutions of 8 per cent or greater concentration, or dextrose solutions of 6 per cent or greater concentration, are hypertonic and may act as hydragogue cathartics.

Skole (90) claimed, on the basis of case studies, that up to 6 teaspoons of β -lactose per day given with evaporated milk and water shortened diarrheal spells. He offered no explanation of his own, but reviewed the literature for and against the use of lactose in intestinal disturbances of infants. Kendall (47) previously had suggested that adding lactose to cow's milk might decrease the incidence of summer diarrhea of young children. He based this idea on the alteration of the mixed type intestinal flora of the artificially-fed infant's stool to the strictly fermentative type predominant in the normal nursing's stool.

SOME EFFECTS OF LACTOSE IN THE DIET OF CHICKENS

Considerable use is made of milk and milk products in poultry feeding. For this reason, the effects of various amounts of milk sugar in the diet of domestic fowl has important practical significance.

Several investigators have fed milk, lactose and milk products to chickens and noted the effects. Shaw (87) concluded from his finding that Barfoed's reagent was not reduced by the intestinal contents of lactose-fed chicks, that lactose was not hydrolyzed in the digestive tract of the fowl. He also observed that chickens fed from hatching on milk alone died after 3 days. Autopsy revealed intense inflammation of gastro-intestinal mucous membranes. Hamilton and Card (38) observed similar irritation of the mucosa when chickens were fed lactose mixed with a moist mash. Diarrhea resulted when over 2 g. of lactose per day were fed. However, up to 8 g. per hen per day were utilized fairly completely, and therefore, the lactose present in the amount of milk, whey or butter milk normally eaten (100-200 ml.) by chickens would be well utilized. It is interesting to note the observation that 8 g. per hen per day represented the approximate upper limit of lactose that the chickens would eat voluntarily under the conditions of their experiment. Plimmer and Rosedale (71), feeding rather high levels of lactose in "secwa" (a dried whey product), did *not* observe any diarrhea.

Beach (5) observed that the pH of the cecal contents of chickens changed from a normal range of pH 6.9 to 7.4 to an acid range of pH 4.4 to 5.6, when he fed lactose alone or in milk products or added to *L. acidophilus* milk. Beach and Davis (6) confirmed this observation of the lowering of cecal pH by lactose. They noted dampness of the litter resulting from watery droppings in the chick pens where lactose or non-fat dry milk solids had been fed. Kline *et al.* (48) also reported both catharsis and the lowering of the pH of the intestinal content when up to 40 per cent of lactose was fed. The inadvisability of adding 40 per cent of the sugar to rations for day-old chicks was noted. The large amount of fluid material found in the intestine and ceca was stated by Kline *et al.* to be "due undoubtedly to the greater water consumption." Probably this statement is partially true, but there may be other factors contributing to the distention of the ceca and the fluidity of their contents. Ashcraft (2) observed marked lowering of cecal pH and some lowering of large intestinal pH by lactose and milk products rations. On such rations the cecal horns were dilated to two or three times the normal size, and contained a yellow, frothy mass of material, while on milk-free rations the cecal content was brown and firm. A ration containing 20 per cent of lactose produced no diarrhea, but rations of dry butter milk, nonfat dry milk solids or dry whey calculated to contain about 20 per cent of lactose produced varying degrees of diarrhea.

THE EFFECTS OF WHEY AND SEPARATED MILK ON GASTRO-INTESTINAL
MOTILITY IN MAMMALS

The purgative action of whey probably has been known for a long time. There are some rather recent references to this laxative property for the human,

rat, pig and chicken. Renaud (75) noted slight diarrhea about 2 hours after ingestion of 200 ml. of whey by a fasting human subject. Spindler (93) reported a "copious diarrhea" beginning within a few hours after large amounts (2-8 gallons) of separated milk were fed as the sole food to swine. Frothy feces appeared after about 3 days of diarrhea. In the preceding section, it may be recalled that Ashcraft (2) also noted frothiness of cecal contents of lactose-fed chickens. He also noted more severe diarrhea in chickens fed a dried whey product than in chickens fed other rations containing the same amount of lactose. Fisher (30) obtained preliminary evidence to indicate that lactose was not the sole factor causing diarrhea when whey was fed. On the other hand, the salt content of whey was not the sole factor responsible. These results, while suggestive, are not conclusive, since only a small number of experimental animals was employed. However, the recent work of Daniel and Harvey (19) also indicated that the soluble salts of whey increase the tendency toward diarrhea which exists on lactose rations.

THE MECHANISM OF LACTOSE-INDUCED LAXATION

The mechanism by which lactose acts to produce diarrhea is not definitely known. There are several possible mechanisms:

(a) One possibility is that lactose directly irritates the intestinal musculature, thereby stimulating the muscles and resulting in stronger or more frequent contractions. Results obtained by Feldberg and Solandt (29), with isolated intestinal segments from rabbits, indicate that this is not the case. More evidence supporting this observation should be obtained. Rettger and Cheplin (76) noted that when enough lactose (300 g.) was ingested, diarrhea tended to occur and much lactose was excreted in the feces. From this observation they concluded (76, p. 110) that "the excess of lactose in itself caused some intestinal irritation either directly or through the production of more acid than could be neutralized or absorbed as quickly as it is formed."

(b) A second possible mode of action of lactose in producing laxation is that of a hydragogue, that is, a substance which produces a watery purgation. Presumably, hydragogues are effective because, being only slowly absorbed from the intestine, many of their ions or molecules remain within the lumen of the gut and thus raise the osmotic pressure therein above that of the blood. Therefore, water diffuses into the intestinal lumen from the blood. This excess water distends the intestinal walls, thus stimulating muscular contraction and also flushes the intestinal tract. Lactose seems to fit this description of a hydragogue for the following reasons. First, it produces watery purgation. Second, it is absorbed² at only one-third to one-half the rate of dextrose, maltose or sucrose (1), because it is more slowly broken down (97, p. 115). Some lactose may escape digestion in the small intestine and pass into the large intestine (97, p. 114; 76, p. 60, p. 107). The presence of lactose molecules in the intestinal lumen thus could exert

² The term "lactose absorption" has been used in this review to mean hydrolysis and absorption as the monosaccharides, glucose and galactose (unless otherwise stated). Most lactose hydrolysis probably occurs during the process of absorption (31).

the supposed hydragogue action. In support of the possibility that lactose acts in this way is the work of Albertoni (1) in which large dogs, fasted for 48 hours, were given from 67 to 167 g. of lactose in solution. The dogs were killed an hour later, and it was found that considerable lactose and water remained in the intestine, and that the stomach and intestine together contained more liquid than had been given.

In the case of such a hydragogue action, if water passes from the blood into the intestinal lumen, then either dehydration of body tissues or decreased urine volume might be expected. That the action of lactose in producing diarrhea may be due to such a dehydrating action was suggested by Whittier (99). Jarvis (45), commenting on his observations of babies and on Killian's unpublished work with rats, thought that lactose diarrhea might be one cause of the lowered water content of rat tissues which resulted when lactose was fed. In Killian's work lactose was compared with sucrose. The data reported by Whittier *et al.* do not entirely support the work of Killian. From table 2 in the paper by Whittier *et al.* (101) it can be calculated that the percentage moisture of the net body weight was greater in the lactose-fed rat than in the sucrose-fed rat of each pair, with *ad libitum* feeding. However, in two pairs of pair-fed rats killed and analyzed at 774 days of age, this difference in tissue moisture was not evident, as can be calculated from table 4 of the same paper. Mitchell *et al.* (56) found more water in the tissues of rats fed lactose, as compared to tissues of rats fed glucose. Handler (40), judging from studies of hematocrit values and serum protein content of the blood of rats fed 81 per cent lactose rations, found no serious dehydration occurring. Urine volume was three times as great as that of a comparable group of rats fed an 81 per cent sucrose ration. This greater urine volume was the result of excretion of galactose in the urine. The data presented by Mitchell and Isbell (57) show that the volume of urine produced by rats on a ration containing 75 per cent ground beef and 25 per cent lactose is not different from that produced by rats on a 100 per cent ground beef ration. Rocceuzzo (82) found that *less* urine was eliminated by men after taking lactose solutions (hypertonic, hypotonic or isotonic solutions) orally than after taking an equal volume of water. Holt and Kajdi (41) found that feeding rats lactose as the sole article of diet resulted in a greater urine volume, diarrhea and a greater water intake. On the other hand, Ershoff (25) did not report a greater water intake by rats fed lactose as the only food. However, greater water intakes have been reported by others when 87.5 per cent lactose rations were fed to rats (35), and when 40 per cent lactose rations were fed to day-old chicks (48).

Two groups of investigators, Schorer and Laffoon (84) and Epstein and Thompson (24), presented evidence that lactose-sodium citrate solutions were effective in diminishing the so-called "dehydration" weight loss of newborn infants. The sodium citrate was supplied as a hydrating agent, the lactose as a food. The β -lactose-sodium citrate solution was more effective than the α -lactose-sodium citrate solution in this respect. Also, the β -lactose solution alone was

more effective than the α -lactose solution, but neither sugar alone was as effective as either of the lactose-sodium citrate combinations. This evidence seems to indicate that the sodium citrate was the main hydrating agent involved, while the lactose effect on birth weight decline may be due to its value as a hydrating agent, or as an energy source. The hydrating or dehydrating action of lactose therefore is not yet definitely established.

Hurst (44 p. 331) was of the opinion that sweet milk had practically no effects on bowel movements, although many persons considered milk to be constipating. Much more recently, Hosoi *et al.* (42) have stated that milk should not be given if it is desired to prevent bowel movements, because milk leaves a large residue in the terminal ileum. Their experiments, which were performed with dogs, indicate that when a small residue is desired, any common food sugar *except* lactose may be included in the low residue diet. Milk and lactose were predominant among the foods which left both a large moist residue and a large dry residue. This copious dry residue suggested to these investigators also that lactose, due to poor absorption by the mucosa, might act by increasing the fluid content of the intestine and thus produce laxation in the same manner as does magnesium sulfate. A solution having a lactose concentration equal to that of milk passed very rapidly through the small intestine. But, in general, when lactose was fed with other foods, the rate of passage of the residues through the small intestine was not increased, while the total residue excreted was increased. Childrey *et al.* (15) later reported that lactose given 1 day interfered with the digestion of foods eaten both on the same day and on the following day. Mitchell *et al.* (56) have also noted the impaired digestion of organic nutrients resulting from lactose feeding. Outhouse *et al.* (68) showed that the fecal pellets of lactose-fed rats had a significantly greater dry weight than the fecal pellets of litter mates fed different rations. Mitchell and Isbell (57) also found that heavier feces (on the wet basis) were obtained from lactose-fed rats. Other references to the effects of lactose on the digestibility of organic nutrients and on the amount of feces excreted have been discussed in the section on intestinal motility in infants. The reported evidence thus seems to show rather conclusively that lactose *does* increase the amount of food residue excreted from the intestine.

Koehler *et al.* (50) have suggested that if the failure of lactose (1.5 g. per kg.) to raise the blood sugar level of normal young adults is due to delayed absorption resulting from slow hydrolysis of the lactose, then the rate of hydrolysis of lactose must be very much slower than that of starch. They have suggested further that rapid passage of lactose residues through the intestine may interfere with hydrolysis, even when appreciable amounts of the lactose-splitting enzyme are present, but no experiments were performed to test this possibility. The question of whether the fast rate of passage of lactose is the cause or the effect of the slight hydrolysis of this sugar thus remains to be settled.

Further evidence that the amounts of lactose hydrolyzed are small is furnished by many observations of "pot-bellies" in lactose-fed animals. Mitchell (60) described this pot-bellied appearance in rats of all strains used by her, when 70

per cent lactose rations were fed. Autopsies revealed that the cecum usually was greatly distended and filled with fluid or a semi-solid mass of material. Mitchell noted that the Wistar strain suffered greater cecal distention than the other strains, due mostly to more gas. She reasoned that rats of this strain probably did not absorb as much of the lactose as rats of the other strains. The bloated condition also has been observed by Mitchell *et al.* (56). Other investigators (54, 63, 95) have observed that the ceca of lactose-fed rats were distended and contained a watery, whitish material, which they presumed to be mainly unabsorbed lactose. Ershoff and Deuel (26) reported that rats fed 73.2 per cent lactose diets sometimes had ceca enlarged in size to almost 20 times those of similar rats fed non-lactose rations. Ershoff (25) found that all of his rats fed only lactose had pot-bellies when alive and showed distention of the cecum at autopsy. Riggs and Beaty (79) observed abdominal distention of increasing persistence and severity as the percentage of lactose in the ration was raised from 20 to 25, 30, 40 and 50 per cent. Ashcraft (2) noted that the ceca of lactose-fed chickens were distended two to three times the normal size. It can be seen in table I of the paper by Mitchell and Isbell (57) that the actual wet weight of the cecal content was more than doubled by incorporating 25 per cent of lactose into an all-meat ration. Similarly, table 2 in the paper by Whittier *et al.* (101) shows a heavier intestinal content as a result of lactose feeding.

(c) Possibly lactose acts to produce diarrhea indirectly through the alteration of the intestinal flora to an aciduric type. This aciduric flora might then produce diarrhea in any one or more of several possible ways. That the flora alteration is not the primary cause of the diarrhea is indicated by the rapid onset of the diarrhea after lactose feeding (26), while the complete flora transformation requires from 3 to 6 days (76, p. 17; p. 76).

(d) Another way that lactose may act is by changing the pH of the intestinal contents enough to stimulate the intestinal muscles directly. Robinson (80) has shown that laxatives, including lactose, produce a lowered pH in the feces of men. Others (2, 5, 6, 14, 43, 48) have demonstrated a lowered pH in the intestinal contents of various species when lactose was fed. Robinson and Duncan (81) reported that the degree of pH lowering by lactose was dependent upon the character of the diet. A much greater effect was produced by a vegetable diet than by a meat diet, even though each diet contained the same percentage (25 per cent) of lactose. The possible relationship of the change in intestinal flora to the change in acidity has been investigated extensively (14, 43, 51, 73, 76, 98). However, Beach (5) has shown that, in the chicken at least, the initial pH change occurred very quickly (2 to 2.5 hours) after lactose feeding before much change in the flora could have occurred. Therefore, the initial pH change associated with lactose ingestion probably is not the result of the flora change. It is possible that the pH change is responsible for the laxation. However, it seems more probable that the laxation is responsible for the initial pH change, for the increased rate of passage of residues would be expected to interfere with the neutralization and absorption of acids (37).

(e) Bergeim *et al.* (7) have shown that lactose maintains a positive oxidation-reduction potential in the intestine. Of the other foods tested, only raw apple possesses in common with lactose the ability to maintain a positive potential. The possible relation of this observation to lactose diarrhea remains obscure, but it is interesting to note that raw apple is considered by many to be a good bowel regulator.

(f) There is one more conceivable way in which lactose may produce its laxative effects. It has been shown repeatedly that the laxative action is due to the lactose molecule *per se*, and not to its hydrolysis products. When a large amount of lactose is ingested, a small part of the disaccharide may be absorbed into the blood stream without being hydrolyzed in the process (97, p. 115). Such minute amounts of unsplit lactose conceivably may act from the blood on the nerve supply of the intestine to increase muscular activity. Such a theory has been proposed (44, pp. 349-350) for the mode of action of saline purgatives. However, this possibility seems unlikely in the case of lactose, since diarrhea apparently has not been reported after lactose injections into the blood stream, but perhaps no observations were made to determine whether or not diarrhea had occurred following the lactose injections. Furthermore, in the case of *injected* lactose, the rapid renal elimination of the sugar would be expected to prevent a prolonged stimulating effect, while in the case of *ingested* lactose, the continuous absorption of small amounts of the unsplit lactose molecule might be expected to exert a prolonged action.

SOME EFFECTS OF OTHER DIETARY CONSTITUENTS ON LACTOSE-INDUCED DIARRHEA

The work of several investigators indicates that the kind and amount of salts ingested with lactose affects the rate of lactose hydrolysis and the incidence and severity of diarrhea. Mitchell *et al.* (61) fed rats adequate diets containing 60 per cent lactose and the Osborne and Mendel salt mixture. This mineral mixture supplied 0.5 per cent calcium. Additional calcium (0.5 or 1 per cent) was added in the form of a different salt for each experiment. The phosphate, carbonate, citrate and levulinate of calcium at both levels gave good growth, the same degree of galactemia and incidence of galactose cataract, *but less diarrhea, in general*, than the 60 per cent lactose ration without added salts. Calcium lactate at the 0.5 per cent level gave similar results, but at the 1 per cent level it tended to decrease diarrhea, yet produced toxic effects. Both the 0.5 and the 1 per cent levels of calcium gluconate gave different results. Poor survival, toxic symptoms, weight loss, low blood sugar and *no* galactose cataract were observed together with diarrhea of a severe nature, especially when the 1 per cent level of calcium gluconate was fed. Sodium gluconate gave similar but more severe results than calcium gluconate. Calcium gluconate apparently did not inhibit the absorption of glucose or galactose or the digestion and absorption of sucrose, dextrin or starch. Growth efficiency was as good with gluconate as without, when glucose and galactose were fed. Therefore, it was thought that the gluconate radical somehow interfered with lactose utilization, perhaps by inhibiting lactase

enzyme activity. Most of the rats fed the lactose ration plus calcium or sodium gluconate showed as severe diarrhea after several weeks as at first; thus adaptation generally was prevented by the gluconates. There were a few rats which survived the feeding of lactose plus gluconate, showing improved growth rate and efficiency of gain in spite of the persistent diarrhea. In these inexplicable cases, the growth recovery indicates the occurrence of adaptation while the continuing diarrhea contraindicates such an occurrence.

Coryell and Christman (17) have presented evidence to show that calcium chloride given with lactose by stomach tube to fasted rats decreased the hydrolysis of the lactose and also decreased the absorption of the galactose. It has been shown by Nordbö (66) that lactose combines with calcium and magnesium to form non-ionized compounds. Nalder (64) thought that this combination might explain the interference of calcium with lactose hydrolysis and galactose absorption.

Riggs and Beaty (79) found no difference in growth, food utilization or occurrence of diarrhea when litter-mate rats were fed a 50 per cent lactose diet containing 3.5 or 7 per cent of a salt mixture (U.S.P. XII salt mixture #2).

The salts of whey, as compared to an equal percentage (13 per cent) of the salts of McCollum's salt mixture, were found by Fisher (30) to intensify diarrhea on a 41 per cent lactose ration. Daniel and Harvey (19) reported that 50 per cent lactose rations containing whey protein produced a more persistent diarrheal condition when the soluble ash of the whey remained with the protein than when this ash was removed by dialysis.

Boutwell *et al.* (10) reported poor outward appearance and the occurrence of diarrhea in rats during the first 6 to 12 days of feeding a ration containing 48 per cent lactose and 28 per cent corn oil. A similar ration containing 28 per cent butter fat in place of the corn oil gave better growth, larger feed intake, and good outward appearance. Boutwell *et al.* (11) later reported less diarrhea among rats fed the ration containing 48 per cent lactose and 28 per cent butter fat than among rats fed the ration containing 48 per cent lactose and 28 per cent corn oil. On the other hand, Deuel *et al.* (21) reported no difference in nutritive value of butter fat and vegetable fats when fed to rats in a ration with 70.6 per cent of nonfat dry milk solids and vitamin supplements. These workers eliminated from the experiment any litter of which two or more members developed diarrhea. It would appear that if butter fat prevents the occurrence or reduces the severity of lactose-induced diarrhea, then a physiological difference between the food fats in question has been demonstrated. Perhaps this point should be considered more carefully in work of this sort. In a later experiment, Ershoff and Deuel (27) studied the length of survival of rats fed diets of 70 per cent carbohydrate and 30 per cent fat. Survival was longer with sucrose than with lactose. The kind of fat fed did not influence significantly the length of survival on the sucrose rations, but on the lactose rations butter fat or margarine allowed significantly longer survival than did corn oil or cottonseed oil.

Rojas *et al.* (83) found that the absence of fat from the diets of dairy calves did not interfere with the utilization of the amounts of lactose usually consumed

in a separated milk ration. As noted previously, doubling the amount of lactose in the separated milk produced diarrhea. However, no study was made of the effect of doubling the lactose content of *whole* milk fed to calves.

Ershoff and Deuel (26) observed severe diarrhea and death of weanling rats fed a 73.2 per cent lactose ration. Each rat was given 800 mg. of corn oil per day. The average survival time for rats of the Long-Evans strain fed β -lactose was 4.9 days. When 10 per cent of lard, oleo or butter fat was fed, the average survival times were 2.4, 3.0 and 3.2 days, respectively. Mitchell (59) fed rats rations containing 18 per cent of butter fat and varying amounts of lactose. At the 60 and 45 per cent lactose levels, growth was poor, while at the 30 per cent level it was practically normal. However, Riggs and Beaty (79) reported slower growth in the early part of an experiment in which rats were fed diets containing 5 per cent fat and 30 per cent lactose. These rats recovered and weighed as much after 12 weeks as rats fed the basal cornstarch ration. That the difference in growth at the 30 per cent lactose level was perhaps due to the higher fat content of Mitchell's diets was suggested by Riggs and Beaty. Their observation of rapid growth and only slight diarrhea in rats fed dried whole milk (27.5 per cent fat, 37.5 per cent lactose) where the lactose intake was about 33 to 34 g. per kg. per day supports this suggestion. Such large lactose intakes caused severe diarrhea on the 5 per cent fat ration. Thus apparently the favorable effect of larger amounts of fat was due not to a reduction in lactose intake, but rather to an improvement in lactose utilization. However, Riggs and Beaty (79) did not think it likely that fat directly affected the hydrolysis of lactose. Such a conclusion is supported by the work of Coryell and Christman (17). They showed that vegetable fat (Mazola) given with lactose by stomach tube did not alter significantly the amount of lactose hydrolysis or of galactose absorption. Nieft and Deuel (65) also reported that corn oil did not alter lactose hydrolysis, but contrary to the results of Coryell and Christman (17), they reported that galactose absorption was delayed by fat. These contradictory results as regards galactose absorption perhaps may be explained by differences in the methods used.

Ershoff and Deuel (26) tried adding various supplements to the 73.2 per cent β -lactose diet which they fed to weanling rats of the Long-Evans strain. The average survival time for such rats fed the β -lactose ration without supplements was 4.9 days. The survival time was lengthened to 7 ± 2 days by feeding 10 per cent of dried mammalian liver, and to 11 ± 3 days by feeding dried tuna liver. The rats fed these supplements gained weight the first 5 to 7 days, then lost weight and showed the typical β -lactose syndrome, including severe diarrhea. Other supplements (lard, oleo, butter fat, cream, wheat germ oil, biotin, brewers' yeast) were ineffective in lengthening survival time.

THE USE OF LACTOSE WITH AND WITHOUT *L. ACIDOPHILUS* FOR TREATING CONSTIPATION AND TRANSFORMING THE INTESTINAL FLORA

Lactose has been used extensively in conjunction with *L. acidophilus* milk for treating constipation of human adults, making difficult any evaluation of the

effect of the sugar alone. However, a few investigators have employed the sugar alone. Traube (96) recommended 9 to 15 g. of lactose in 0.25 l. of warm milk, taken in the morning on an empty stomach. Albertoni (1) recommended 20 g. of lactose taken in water. Boros (8, 9) reported that 16 out of 20 cases of constipation selected at random were benefited by lactose therapy and a prescribed diet. Two tablespoons of lactose in one-half glass of cold water were given twice a day. This is equivalent to about 38 g. per day.³ In some cases a few days were required before results were observed. The doses of sugar could be reduced or cut out entirely after normal bowel habits were established.

Metchnikoff (55) first recommended the use of Bulgarian sour milk as a means of prolonging life, reasoning from observations of the long life of some Bulgarian peasants and from some rather meager experimental results obtained in his laboratory. His theory was that *Lactobacillus bulgaricus* became implanted in the intestine to the exclusion of harmful bacteria. However, several investigators have shown that *L. bulgaricus* does not survive in the digestive tract.

Rettger and Cheplin (76) have given a complete review of the literature on the subject of sour milk organisms in the intestine and have given a detailed account of their work in the field. They showed that *L. acidophilus* and *L. bifidus* (which may be identical organisms) can be stimulated to grow and to form a large part of the intestinal flora by feeding lactose, dextrin or massive doses of cultures of the organism. The probable explanation of the stimulating action of lactose and dextrin alone among the carbohydrates is that only these two carbohydrates reach the large intestine in large amounts and there provide a suitable food source for the *L. acidophilus* organism. These workers found that the intestinal flora of rats usually was transformed completely to an aciduric type by feeding 2 g. of lactose or dextrin per day, by 2 ml. of a culture of *L. acidophilus* or by 1 g. lactose or dextrin with 1 ml. of the culture. In a similar manner, the human intestinal flora usually was transformed completely by 300 to 400 g. of lactose or dextrin, by 300 ml. of *L. acidophilus* culture or by 150 g. of lactose or dextrin and 150 ml. of the culture. Also, in man, the flora transformation was effected by 1000 ml. of a 2-to-24 hour culture of *L. acidophilus* in milk or by 500 ml. of such a milk culture plus 100 g. of lactose.

It is interesting to note that 300 g. of lactose, the dose usually needed for complete transformation of the flora in man, corresponds to the approximate level where a tendency toward diarrhea was observed by these investigators. Others (1, 53, 80, 96), however, have observed diarrhea in man after ingestion of somewhat smaller amounts of lactose. Rettger and Cheplin were aware of a possible direct effect of lactose on intestinal motility. However, Rettger *et al.* (77) considered that the relatively large amounts of lactose which they employed with *L. acidophilus* milk for successfully treating some types of constipation constituted a pabulum for the organisms, rather than having any direct benefit.

³ This figure of 38 g. per day was obtained by assuming that one level tablespoon of lactose weighs $\frac{1}{2}$ oz. Thus the four-tablespoon intake would correspond to $1\frac{1}{2}$ oz., or 37.8 g.

Kopeloff and Cheney (52) obtained relief from chronic constipation by giving 1000 ml. of *L. acidophilus* whole milk and 300 g. of lactose per day. They also treated diarrhea with the milk, but omitted the lactose because of its known diarrheal effects. Kopeloff (51, p. 109) satisfied himself by numerous experiments that the effects of the *L. acidophilus* milk were mainly bacteriological, rather than physical or chemical. He (51, p. 52) realized the benefits in treating constipation, of using lactose with the milk and of continuing the use of lactose after discontinuing the milk treatment for prolonging the beneficial effects. He postulated (51, p. 53) that the benefits might be due either to lactose being a suitable pabulum for *L. acidophilus* in the intestine, or to lactose *per se* relieving constipation. Apparently, he believed the first possibility to be the case, since the use of lactose alone did not produce as uniformly good results as its use with *L. acidophilus* milk, and the addition of lactose to *L. acidophilus* milk produced no great improvement over the use of *L. acidophilus* milk alone.

On the other hand, Clark and Perry (16) got much better results when lactose was given with *L. acidophilus* milk than when the milk was given alone. They reported less constipation in 80 per cent of their patients given 1 quart of *L. acidophilus* milk per day with 75 g. of additional lactose, while only 30 per cent of the patients given 1 quart of the milk alone were less constipated, and 40 per cent were more constipated. It is to be noted that these workers used much smaller amounts of lactose than did Rettger *et al.* (77) and Kopeloff (51). A possible explanation for the failure of Clark and Perry to get better results with the milk alone might be that their milk perhaps did not contain sufficient numbers of viable organisms. Cruickshank (18) thought that the effectiveness of *L. acidophilus* milk was due to a combination of viable organisms and the unfermented lactose in the milk.

The results of these various investigators seem to conflict. It is difficult to ascertain from a study of their work whether or not lactose alone has any therapeutic value in treating constipation, but the indications are that it possibly has some such value. It does seem, from a consideration of the work which has been done, that lactose has at least indirect benefits in constipation through the encouragement of the *L. acidophilus* organisms. According to Kopeloff (51, pp. 115-116) these organisms may produce their benefits in several possible ways:

“(1) *L. acidophilus* may secrete enzymes which act directly upon the intestinal mucosa to stimulate peristalsis. (2) *L. acidophilus* may secrete or excrete substances toxic to putrefactive organisms. (3) *L. acidophilus* may change the hydrogen ion concentration of the intestinal tract to such a degree as to inhibit the growth of putrefactive organisms. (4) *L. acidophilus* may change the hydrogen ion concentration of the intestinal tract to such a degree as to stimulate peristalsis directly. (5) *L. acidophilus* may produce lactic acid of chemical structure different from inactive lactic acid or that produced by other microorganisms usually present in the intestine which may directly stimulate peristalsis.”

It has been suggested by Smith (92) that a high-lactose diet alone is not so effective in transforming the flora in the intestine of man as in the intestine of

the rat. This relative ineffectiveness of lactose alone partially may explain the variability of results which have been obtained in attempted therapeutic applications of *L. acidophilus* and lactose feeding in man. Smith (92) also observed that the transformation of the intestinal flora was aided by giving laxatives with lactose. He reasoned that the laxatives probably increased the rate of passage and hindered absorption of the lactose, allowing larger amounts to reach the lower bowel, there to stimulate the aciduric organisms. This observation confirms the theory of the Yale workers (76) that lactose can stimulate *L. acidophilus* because such large amounts reach the lower bowel unabsorbed.

Mitchell (59), working with rats, observed that increasing percentages of food sugar lost in the feces and increasing percentages of *L. acidophilus* paralleled increasing percentages of lactose in the ration. *L. acidophilus* constituted 50 per cent or more of the flora when 30 per cent or more of lactose was fed. But at 45 and 60 per cent levels of lactose feeding, poor growth of the rats was obtained, even though a higher percentage of *L. acidophilus* was found in the flora. In a companion paper, Mitchell (58) found that the high percentage of *L. acidophilus* lasted only while diarrhea accompanied lactose feeding. On a 60 per cent lactose ration, the *L. acidophilus* count dropped from 80-95 per cent of the total flora to 13-15 per cent of the flora when intestinal stasis was produced by giving a few grains of "tannalbin" daily as an astringent. The diarrhea stopped in a few days and the growth rate changed from a decreasing rate to an increasing rate in 2 weeks. When Dragstedt *et al.* (22) produced intestinal stasis by reversing a 12-inch segment of intestine or by ligation of the intestine, the flora was proteolytic in character, irrespective of the nature of the diet.

ADAPTATION TO LACTOSE FEEDING

An animal may adapt to lactose feeding; that is, the diarrhea may become less severe, may occur less commonly or may even cease entirely if the animal continues to ingest rather large amounts of lactose over a period of time. Several investigators have reported adaptation to lactose feeding in rat experiments. Whittier *et al.* (101) noted that rations containing 45 and 63.5 per cent of lactose produced diarrhea and retarded growth in weanling rats, but that these rats recovered in about 3 weeks. Mitchell and Dodge (62) observed that the diarrheal condition of young rats fed 70 per cent lactose diets subsided in time, but the fecal pellets continued to be softer than usual. Morgan *et al.* (63) reported cessation of diarrhea after a week or two in rats fed a 65 per cent lactose ration. Mitchell *et al.* (61) found that young rats fed rations containing 50 per cent or more of lactose outgrew the lactose diarrhea in a few weeks, except when the ration contained 0.5 per cent of calcium gluconate or of sodium gluconate. When either of these salts was included in the diet, the severe diarrhea in most cases persisted after several weeks. These investigators implicated the gluconate radical; and since this radical did *not* inhibit glucose or galactose absorption, they thought that it must interfere with lactose hydrolysis, possibly by some mechanism of "competitive inhibition" of lactase action. Riggs and Beaty (79)

recently have shown again that rats, through the repeated daily ingestion of lactose, develop the ability to utilize lactose without suffering from diarrhea.

Possibly the mechanism of adaptation to lactose feeding is an increased production of the lactase enzyme in the intestinal mucosa, but there is no evidence in the literature to support this possibility. Mitchell (58) noted that the aciduric intestinal flora produced by lactose feeding tended to revert to the putrefactive type after a time if lactose ingestion was continued. This observation may indicate that smaller amounts of unhydrolyzed lactose were reaching the lower levels of the bowel, and therefore that lactose-hydrolyzing ability had been increased in some way. One possibility is that the mucosal cells lining the intestine had been stimulated to produce larger amounts of lactase enzyme. The previously cited observations of decreased incidence and severity of lactose-produced diarrhea after some time on lactose diets may indicate the same mechanism of increased lactose hydrolysis due possibly to increased lactase production. Therefore, a consideration of the properties and occurrence of this enzyme is important.

Florey *et al.* (31) reviewed some of the literature on the subject of lactase and concluded that most of the lactase action is due to the mucosal cells rather than to the intestinal juice, and therefore, that most lactose digestion occurs during absorption.

Plimmer (70) used a copper reduction method to show that lactase was present in the intestines of young mammals and persisted in older mammals of some species, but not of other species. The guinea pig, for example, lost its intestinal lactase activity in 5 weeks after birth, while lactase activity continued to some extent in the adult dog and rabbit. Experiments showed that lactase production was not increased by feeding milk and lactose.

Plimmer and Rosedale (72) and Hamilton and Mitchell (39) demonstrated the presence of the enzyme in the crop of the chicken, but not in the proventriculus, pancreas or intestine.

Cajori (12) could not find lactase in intestinal juice and thought that either lactose must be absorbed in part without being hydrolyzed, or else the enzyme action was carried on in the mucosal cells. Later he (13) reported lactase in the jejunal and duodenal mucosa and in the liver. The jejunal mucosa had a lactase activity 10-30 per cent greater than that of the duodenal mucosa. The liver lactase activity was very small. The optimum pH range for dog intestinal lactase was 5.4 to 6.0. Glucose inhibited lactase action, for in the presence of glucose 40 per cent less lactose was hydrolyzed in 22 hours. Galactose had practically no effect.

Ershoff and Deuel (26) reported a strain difference with respect to average survival time and severity of diarrhea in rats fed a 73.2 per cent lactose ration. They proposed that this difference might be due to a difference in intestinal lactase activity. The University of Southern California strain was not as severely affected as the Long-Evans strain, indicating possibly greater lactose hydrolysis. Species, age and individual differences in susceptibility to lactose feeding have

been discussed previously, and these also may be due to differences in lactase activity.

Koehler *et al.* (50) expressed doubt that slight lactose hydrolysis necessarily was the cause of the rapid rate of passage of lactose. They theorized that the rapid passage might be the cause of the slight hydrolysis, even when considerable amounts of lactase were present, since there would be little time for enzyme action. If this were so, then perhaps some method of action of lactose other than a hydragogue effect would be indicated.

SUMMARY

The literature dealing with the effects of lactose on the rate of movement of food residues in the gastro-intestinal tract has been reviewed. Species, strain, age and individual differences in susceptibility to lactose-induced laxation have been reported.

A lactose feeding level of 20 per cent of the diet often induces diarrhea in weanling rats of the Sprague-Dawley strain. When lactose in sufficient amounts to produce diarrhea is fed, the animals exhibit pot-bellies, slow growth and reduced feed consumption. These symptoms become progressively more severe and persistent as the percentage of lactose in the ration is increased. The lowest levels of lactose feeding which will initiate diarrhea in species other than the rat have not been carefully determined, although the occurrence of diarrhea has been reported in all species studied.

Early death in rats fed rations containing 61.8 to 100 per cent lactose has been reported by several investigators.

The lactose linkage apparently is responsible for the diarrhea and some of the other symptoms noted when moderate to large amounts of lactose are fed.

The literature reports, based generally upon case studies rather than upon carefully controlled experiments, indicate that more than about 12 to 19 per cent of lactose in the formula is apt to cause diarrhea in human infants. This may be compared with 10 to 15 per cent for puppies, and 20 per cent for weanling rats. Other reports suggest that smaller amounts of lactose may be valuable in controlling or preventing diarrheal conditions.

The available evidence indicates that lactose in large amounts (20 per cent or more of the ration) tends to produce diarrhea in chickens and to lower the pH of the contents of the ceca markedly and of the large intestine somewhat less. The ceca are distended by a frothy liquid material. Water consumption is increased. When lactose constitutes about 40 per cent of the ration of young chicks, extreme inflammation of intestinal mucous membranes may result, and death often may ensue.

The evidence seems to show that the purgative action of large amounts of skim milk and whey is due partly, but not entirely, to lactose.

Lactose may act to produce diarrhea in several possible ways, for example:

(a) Direct stimulation of intestinal muscles. (b) Hydragogue action; interfering with absorption of water and organic nutrients. (c) Alteration of intes-

tinal flora to an aciduric type, the aciduric flora then acting in several different possible ways. (d) Alteration of pH enough to stimulate the intestinal musculature. (e) Maintenance of a positive oxidation-reduction potential. (f) Stimulation of intestinal muscle through its nerve supply by small amounts of unhydrolyzed lactose in the blood.

Of these possibilities, the first, third and sixth are contraindicated by the available evidence, while the fourth appears to be an effect rather than a cause of the diarrhea. The possible relation, if any, of the positive oxidation-reduction potential to lactose diarrhea is not clear. Thus, at the present time, the second possibility, that of a hydragogue type of action, seems most plausible. However, some evidence does not support the idea that tissue dehydration is accomplished by persistent lactose diarrhea. Dehydration would be expected if a continuous hydragogue action were involved, unless water consumption were increased or urine volume decreased. It has been shown that water intake sometimes is increased, at least on rations consisting solely or largely of lactose. On such high-lactose rations, urine volume also increases as a result of the urinary excretion of galactose, whereas urine volume decreases in many diarrheal conditions. However, the work of one investigator does indicate *decreased* diuresis when lactose is given. Water balance experiments at lower lactose feeding levels seem to be desirable in order to better evaluate the possibility of a hydragogue type of action.

Other dietary constituents seem to influence the occurrence, severity and persistence of lactose-produced diarrhea. Certain calcium salts may decrease the severity of the diarrhea to some extent, while both calcium gluconate and sodium gluconate seem to increase the severity of the diarrhea and prevent adaptation to lactose feeding. It has been demonstrated that calcium chloride decreases the rate of lactose hydrolysis and galactose absorption. A certain portion of the salts of whey intensifies and prolongs lactose diarrhea. Increasing the amounts of fat in the diet tends to decrease the diarrhea, and there is some indication that butter fat may be more valuable in this respect than certain other food fats. The feeding of liver may postpone but not prevent the appearance of lactose-feeding symptoms on a high-lactose diet.

Lactose has been used successfully in conjunction with and for prolonging the effect of *L. acidophilus* in transforming the intestinal flora to a predominantly aciduric type. The use of lactose without *L. acidophilus* seems to have definite therapeutic possibilities as yet little investigated. Evidence indicates that the permanent transformation of the flora by lactose depends upon sufficient amounts of the sugar continually arriving in the lower bowel, for the flora reverts to the normal mixed type when lactose feeding is discontinued or when intestinal stasis is produced.

Adaptation to lactose feeding is evidenced by the diarrheal condition becoming less conspicuous or disappearing completely.

Lactase occurs in the mucosal cells of the intestines of mammals, but not to any extent in the intestinal juice. Therefore, lactose hydrolysis must be mainly an

intracellular process, occurring during absorption. Since lactose diarrhea probably is the result of slight lactose hydrolysis and the resulting hydragogue effect, then the species, strain, age and individual differences in the severity of lactose feeding symptoms might be due to differences in intestinal lactase activity. Also, it seems likely that adaptation to lactose feeding is brought about by increased intestinal lactase activity. The results of one early investigator indicate that increased lactase activity does *not* result from continued milk or lactose feeding, but these experiments have not been confirmed.

REFERENCES

- (1) ALBERTONI, P. Manière de se comporter des sucres et leur action dans l'organisme. Arch. ital. biol., 15: 321-343. 1891.
- (2) ASHCRAFT, D. W. Effect of Milk Products on pH of Intestinal Contents of Domestic Fowl. Poultry Sci., 12: 292-298. 1933.
- (3) BARENBERG, L. H., AND ABRAMSON, H. Effects of Large Amounts of Milk Sugar on the Stools and Nutrition of Infants. Arch. Pediat., 47: 1-7. 1930.
- (4) BARKER, L. F. The Diet in Typhoid Fever. J. Am. Med. Assoc., 63: 929-931. 1914.
- (5) BEACH, J. R. The Effect of Feeding *Bacillus acidophilus*, Lactose, Dry Skim Milk, or Whole Milk on the Hydrogen-ion Concentration of the Contents of the Ceca of Chickens. Hilgardia, 1(8): 145-165. 1925.
- (6) BEACH, J. R., AND DAVIS, D. F. The Influence of Feeding Lactose or Dry Skim Milk on Artificial Infection of Chicks with *Eimeria avium*. Hilgardia, 1(8): 167-181. 1925.
- (7) BERGEIM, O., KLEINBERG, J., AND KIRCH, E. R. Oxidation-reduction Potentials of the Gastrointestinal Tract. J. Bact., 49: 453-458. 1945.
- (8) BOROS, E. The Use of Lactose in the Treatment of Constipation. Med. J. and Record, 134: 131-135. 1931.
- (9) BOROS, E. Milk Sugar in the Treatment of Intestinal Toxemia with Special Reference to Constipation. Med. J. and Record, 137: 140-144. 1933.
- (10) BOUTWELL, R. K., GEYER, R. P., ELVEHJEM, C. A., AND HART, E. B. Effect of Flavor on the Nutritive Value of Fats. Proc. Soc. Exptl. Biol. Med., 55: 153-155. 1944.
- (11) BOUTWELL, R. K., GEYER, R. P., ELVEHJEM, C. A., AND HART, E. B. Studies on the Interrelation of Fats, Carbohydrates, and B-vitamins in Rat Nutrition. Arch. Biochem., 7: 143-157. 1945.
- (12) CAJORI, F. A. The Enzyme Activity of Dogs' Intestinal Juice and its Relation to Intestinal Digestion. Am. J. Physiol., 104: 659-668. 1933.
- (13) CAJORI, F. A. The Lactase Activity of the Intestinal Mucosa of the Dog and Some Characteristics of Intestinal Lactase. J. Biol. Chem., 109: 159-168. 1935.
- (14) CANNON, P. R., AND MCNEASE, B. W. Factors Controlling Intestinal Bacteria. The Influence of Hydrogen-ion Concentration on Bacterial Types. J. Infectious Diseases, 32: 175-180. 1923.
- (15) CHILDREY, J. H., ALVAREZ, W. C., AND MANN, F. C. Digestion. Efficiency with Various Foods, and Under Various Conditions. Arch. Internal Med., 46: 361-374. 1930.
- (16) CLARK, W. E., AND PERRY, M. W. Clinical Value of Acidophilus Milk. Med. J. and Record, 118: 740-742. 1923.
- (17) CORYELL, M. N., AND CHRISTMAN, A. A. The Utilization of Lactose by the Fasting White Rat. J. Biol. Chem., 150: 143-154. 1943.
- (18) CRUICKSHANK, R. The Experimental Transformation of the Intestinal Flora. Brit. J. Exptl. Path., 9: 318-326. 1928.
- (19) DANIEL, F. K., AND HARVEY, E. H. Some Observations on the Nutritional Value of Dialyzed Whey Solids. J. Nutrition, 33: 429-436. 1947.

- (20) DENNETT, R. H., AND CRAIG, J. D. A Comparative Study of Infant Foods. *Med. J. and Record*, **136**: 133-135. 1932.
- (21) DEUEL, H. J., JR., MOVITT, E., HALLMAN, L. F., AND MATTSO, F. H. Studies on the Comparative Nutritive Value of Fats. I. Growth Rate and Efficiency of Conversion of Various Diets to Tissue. *J. Nutrition*, **27**: 107-121. 1944.
- (22) DRAGSTEDT, L. R., CANNON, P. R., AND DRAGSTEDT, C. A. Factors Controlling the Intestinal Bacteria. The Effect of Acute Obstruction and Stasis on Bacterial Types. *J. Infectious Diseases*, **31**: 209-214. 1922.
- (23) DRAGSTEDT, L. R., AND PEACOCK, S. C. Studies on the Pathogenesis of Tetany. I. The Control and Cure of Parathyroid Tetany by Diet. *Am. J. Physiol.*, **64**: 424-434. 1923.
- (24) EPSTEIN, I. M., AND THOMPSON, I. Prevention of Weight Loss in Newborn Infants. *Arch. Pediat.*, **53**: 133-136. 1936.
- (25) ERSHOFF, B. H. Studies on the Nutritive Value of Lactose and Galactose with the Single-Food Choice Method. *Am. J. Physiol.*, **147**: 13-18. 1946.
- (26) ERSHOFF, B. H., AND DEUEL, H. J., JR. Inadequacy of Lactose and Beta Lactose as Dietary Carbohydrates for the Rat. *J. Nutrition*, **28**: 225-234. 1944.
- (27) ERSHOFF, B. H., AND DEUEL, H. J., JR. A Comparison of the Nutritive Values of Fats when Fed Alone or when Fed with Sucrose and Lactose. *Am. J. Physiol.*, **148**: 45-50. 1947.
- (28) EVANS, H. M., AND BURR, G. O. A New Dietary Deficiency with Highly Purified Diets. *Proc. Soc. Exptl. Biol. Med.*, **24**: 740-743. 1927.
- (29) FELDBERG, W., AND SOLANDT, O. M. The Effects of Drugs, Sugars, and Allied Substances on the Isolated Small Intestine of the Rabbit. *J. Physiol. (London)*, **101**: 137-171. 1942.
- (30) FISHER, E. A. The Utilization of Lactose in the Rat and the Pig. (Some Preliminary Observations). *New Zealand J. Sci. Technol.*, **13**: 96-103. 1931.
- (31) FLOREY, H. W., WRIGHT, R. D., AND JENNINGS, M. A. Secretions of the Intestine. *Physiol. Revs.*, **21**: 36-69. 1941.
- (32) FRENCH, R. B., AND COWGILL, G. R. Immaturity of the Organism as a Factor Determining the Favorable Influence of Lactose on the Utilization of Calcium and Phosphorus. *J. Nutrition*, **14**: 383-399. 1937.
- (33) GERSTLEY, J. R. The Influence of Feeding on Certain Acids in the Feces of Infants. Clinical Considerations. *Am. J. Diseases Children*, **40**: 27-45. 1930.
- (34) GERSTLEY, J. R., WANG, C. C., BOYDEN, R. E., AND WOOD, A. A. The Influence of Feeding on Certain Acids in the Feces of Infants. II. The Effect of an Excess of Lactose in Breast Milk and in Modified Cow's Milk, with Observations on a Parenteral Infection. *Am. J. Diseases Children*, **36**: 289-297. 1928.
- (35) GREISHEIMER, R. M., AND JOHNSON, O. H. Glycogen Formation in Rats. III. Diets Containing 87.5 Per cent of the Total Caloric Value in Lactose, Glucose and Sucrose. *Proc. Soc. Exptl. Biol. Med.*, **27**: 769-770. 1930.
- (36) GROSS, E. G. The Effect of Lactose on Calcium-Phosphorus Balance in Dogs. *Am. J. Physiol.*, **80**: 661-667. 1927.
- (37) GROVE, E. W., OLMSTED, W. H., AND KOENIG, K. The Effect of Diet and Catharsis on the Lower Volatile Fatty Acids in the Stools of Normal Men. *J. Biol. Chem.*, **85**: 127-136. 1929.
- (38) HAMILTON, T. S., AND CARD, L. E. The Utilization of Lactose by the Chicken. *J. Agr. Research*, **27**: 597-604. 1924.
- (39) HAMILTON, T. S., AND MITCHELL, H. H. The Occurrence of Lactase in the Alimentary Tract of the Chicken. *J. Agr. Research*, **27**: 605-608. 1924.
- (40) HANDLER, P. The Biochemical Defect Underlying the Nutritional Failure of Young Rats on Diets Containing Excessive Quantities of Lactose or Galactose. *J. Nutrition*, **33**: 221-233. 1947.

- (41) HOLT, L. E., JR., AND KAJDI, C. N. The Nutritional Requirements in Inanition. I. Observations on the Ability of Single Foodstuffs to Prolong Survival. *Bull. Johns Hopkins Hosp.*, **74**: 121-141. 1944.
- (42) HOSOI, K., ALVAREZ, W. C., AND MANN, F. C. Intestinal Absorption: A Search for a Low Residue Diet. *Arch. Internal Med.*, **41**: 112-126. 1928.
- (43) HUDSON, N. P., AND PARR, L. W. Relation of Reaction of Intestinal Contents to Diet and Flora. *J. Infectious Diseases*, **34**: 621-624. 1924.
- (44) HURST, A. F. Constipation and Allied Intestinal Disorders. 2nd Ed. H. Frowde, London, 1919.
- (45) JARVIS, B. W. Milk Sugar in Infant Feeding. *Am. J. Diseases Children*, **40**: 993-999. 1930.
- (46) JEANS, P. C., AND MARRIOTT, W. M. Infant Nutrition. 4th Ed. The C. V. Mosby Co., St. Louis. 1947.
- (47) KENDALL, A. I. Certain Fundamental Principles Relating to the Activity of Bacteria in the Intestinal Tract. Their Relation to Therapeutics. *J. Med. Research*, **25**: 117-187. 1911.
- (48) KLINE, O. L., KEENAN, J. A., ELVEHJEM, A. C., AND HART, E. B. Lactose in Nutrition. *J. Biol. Chem.*, **98**: 121-131. 1932.
- (49) KOEHLER, A. E., AND ALLEN, S. E. The Nutritive Value of Lactose. *J. Nutrition*, **8**: 377-383. 1934.
- (50) KOEHLER, A. E., RAPP, I., AND HILL, E. The Nutritive Value of Lactose in Man. *J. Nutrition*, **9**: 715-724. 1935.
- (51) KOPELOFF, N. *Lactobacillus acidophilus*. The William and Wilkins Co., Baltimore. 1926.
- (52) KOPELOFF, N., AND CHENEY, C. O. Studies on the Therapeutic Effect of *Bacillus acidophilus* Milk and Lactose. (Preliminary communication.) *J. Am. Med. Assoc.*, **79**: 609-611. 1922.
- (53) KOPELOFF, N., AND COHEN, P. Lactose and Intestinal Flora. *J. Am. Med. Assoc.*, **94**: 1983-1985. 1930.
- (54) MANNERING, G. J., ORSINI, D., AND ELVEHJEM, C. A. Effect of the Composition of the Diet on the Riboflavin Requirement of the Rat. *J. Nutrition*, **28**: 141-156. 1944.
- (55) METCHNIKOFF, E. The Prolongation of Life. G. P. Putnam's Sons, New York, London. 1910.
- (56) MITCHELL, H. H., HAMILTON, T. S., AND BEADLES, J. R. The Comparative Nutritive Values of Glucose, Fructose, Sucrose, and Lactose, when Incorporated in a Complete Diet. *J. Nutrition*, **14**: 435-452. 1937.
- (57) MITCHELL, H. K., AND ISBELL, E. R. Intestinal Bacterial Synthesis as a Source of B Vitamins for the Rat. *Univ. Texas Pub.*, Number 4237, Pp. 125-134. 1942.
- (58) MITCHELL, H. S. Comparative Physiological Value of Five Carbohydrates, Based on Growth and Fecal Analysis. *Am. J. Physiol.*, **79**: 537-541. 1927.
- (59) MITCHELL, H. S. Comparative Physiological Values of Different Amounts of Lactose, Based on Growth and Fecal Analysis. *Am. J. Physiol.*, **79**: 542-544. 1927.
- (60) MITCHELL, H. S. Susceptibility of Different Strains of Rats to Nutritional Cataract. *J. Nutrition*, **12**: 447-453. 1936.
- (61) MITCHELL, H. S., COOK, G. M., AND O'BRIEN, K. L. Effect of Several Calcium Salts on the Utilization of Lactose. *J. Nutrition*, **18**: 319-327. 1939.
- (62) MITCHELL, H. S., AND DODGE, W. M., JR. Cataract in Rats Fed on High Lactose Rations. *J. Nutrition*, **9**: 37-49. 1935.
- (63) MORGAN, A. F., COOK, B. B., AND DAVISON, H. G. Vitamin B₂ Deficiencies as Affected by Dietary Carbohydrate. *J. Nutrition*, **15**: 27-43. 1938.
- (64) NALDER, M. E. The Utilization of Lactose by the White Rat. University Microfilms, Publication No. 224. 1940. Microfilm Abstracts, **2**(1): 3. 1941.

- (65) NIEFT, M. L., AND DEUEL, H. J., JR. The Effect of Fat on the Absorption and Utilization of Galactose by the Rat. *J. Biol. Chem.*, **167**: 521-525. 1947.
- (66) NÖRDBO, R. The Concentration of Ionized Magnesium and Calcium in Milk. *J. Biol. Chem.*, **128**: 745-757. 1939.
- (67) OUTHOUSE, J., SMITH, J., MERRITT, L., AND WHITE, F. R. A Comparative Study of the Growth-promoting and Bone-califying Effects of Several Carbohydrates. *J. Nutrition*, **14**: 579-595. 1937.
- (68) OUTHOUSE, J., SMITH, J., AND TWOMEY, I. The Relative Effects of Certain Saccharides and of Vitamin D on Mineral Metabolism of Rats. *J. Nutrition*, **15**: 257-258. 1938.
- (69) PAFFRATH, H., AND SIERING-KAULLA, H. Untersuchungen über die Kohlehydratverdauung des Säuglings. II. Mitteilung. Zuckertoleranzprüfungen an darmgesunden Kindern. *Z. Kinderheilk.*, **48**: 76-85. 1929.
- (70) PLIMMER, R. H. A. On the Presence of Lactase in the Intestines of Animals and on the Adaptation of the Intestine to Lactose. *J. Physiol. (London)*, **35**: 29-31. 1906-7.
- (71) PLIMMER, R. H. A., AND ROSEDALE, J. L. The Rearing of Chickens on the Intensive System. Part II. The Effect of "Good" Protein. *Biochem. J.*, **16**: 19-22. 1922.
- (72) PLIMMER, R. H. A., AND ROSEDALE, J. L. Distribution of Enzymes in the Alimentary Canal of the Chicken. *Biochem. J.*, **16**: 23-26. 1922.
- (73) PORTER, J. R., AND RETTGER, L. F. Influence of Diet on the Distribution of Bacteria in the Stomach, Small Intestine, and Cecum of the White Rat. *J. Infectious Diseases*, **66**: 104-110. 1940.
- (74) PORTER, L., AND DUNN, C. H. Some Studies on Sugars in Infant Feeding. *Am. J. Diseases Children*, **10**: 77-86. 1915.
- (75) RENAUD, M. Sur l'action purgative et laxative du lacto-sérum. Son mécanisme. Le principe des purgations hépato-duodénales. *Comp. rend. soc. biol.*, **98**: 1213-1214. 1928.
- (76) RETTGER, L. F., AND CHEPLIN, H. A. A Treatise on the Transformation of the Intestinal Flora with Special Reference to *Bacillus acidophilus*. Yale Univ. Press, New Haven. 1921.
- (77) RETTGER, L. F., LEVY, M. N., WEINSTEIN, L., AND WEISS, J. E. *Lactobacillus acidophilus* and its Therapeutic Application. Yale Univ. Press, New Haven. 1935.
- (78) RICHTER, C. P. The Nutritional Value of Some Common Carbohydrates, Fats, and Protein Studied in Rats by the Single-Food Choice Method. *Am. J. Physiol.*, **133**: 29-42. 1941.
- (79) RIGGS, L. K., AND BEATY, A. Some Unique Properties of Lactose as a Dietary Carbohydrate. *J. Dairy Sci.*, **30**: 939-950. 1947.
- (80) ROBINSON, C. S. The Hydrogen Ion Concentration of Human Feces. *J. Biol. Chem.*, **52**: 445-466. 1922.
- (81) ROBINSON, C. S., AND DUNCAN, C. W. The Effect of Lactose and the Acid-base Value of the Diet on the Hydrogen-ion Concentration of the Intestinal Contents of the Rat and their Possible Influence on Calcium Absorption. *J. Biol. Chem.*, **92**: 435-447. 1931.
- (82) ROCCUZZO, M. Modifications of Diuresis in Man After the Ingestion of Various Concentrations of Lactose Solutions. *Boll. soc. ital. biol. sper.*, **20**: 5-7. 1945. *Chem. Abstracts*, **40**: 5838^s. 1946.
- (83) ROJAS, J., SCHWEIGERT, B. S., AND RUPEL, I. W. The Utilization of Lactose by the Dairy Calf Fed Normal or Modified Milk Diets. *J. Dairy Sci.*, **31**: 81-87. 1948.
- (84) SCHORER, E. H., AND LAFFOON, F. L. A Clinical Evaluation of Seven Prelactal Feeding Procedures in 962 Consecutive New-Born Infants. *J. Pediat.*, **7**: 613-624. 1935.
- (85) SCHWEIGERT, B. S., MCINTIRE, J. M., HENDERSON, L. M., AND ELVEHJEM, C. A. Intestinal Synthesis of B Vitamins by the Rat. *Arch. Biochem.*, **6**: 403-410. 1945.

- (86) SCOTT, E. M., AND VERNEY, E. Self-selection of Diet. V. Appetite for Carbohydrates. *J. Nutrition*, 34: 401-407. 1947.
- (87) SHAW, T. P. Digestion in the Chick. *Am. J. Physiol.*, 31: 439-446. 1913.
- (88) SISSON, W. R. Experimental Studies of the Intestinal Flora. *Am. J. Diseases Children*, 13: 117-127. 1917.
- (89) SKEGGS, H. R., AND WRIGHT, L. D. Vitamin B Complex Studies with Diets Differing in the Carbohydrate Component. *J. Nutrition*, 32: 375-386. 1946.
- (90) SKOLE, S. M. Lactose in the Treatment of Diarrhea and Vomiting in Infants with Illustrative Case Reports. *Arch. Pediat.*, 50: 395-413. 1933.
- (91) SKOLE, S. M. Evaporated Milk and Beta-Lactose in Infant Feeding. *Med. Record*, 139: 87-90. 1934.
- (92) SMITH, R. P. The Implantation or Enrichment of *Bacillus acidophilus* and Other Organisms in the Intestine. *Brit. Med. J.*, 2: 948-950. 1924.
- (93) SPINDLER, L. A., ZIMMERMAN, H. E., JR., AND HILL, C. H. Preliminary Observations on the Control of Worm Parasites in Swine by the Use of Skim Milk. *Proc. Helminthol. Soc. Wash. D. C.*, 11: 9-12. 1944.
- (94) TALBOT, F. B., AND HILL, L. W. The Influence of Lactose on the Metabolism of an Infant, with Special Reference to Fat, Nitrogen, and Ash. *Am. J. Diseases Children*, 8: 218-227. 1914.
- (95) TEPLY, L. J., KREHL, W. A., AND ELVEHJEM, C. A. The Intestinal Synthesis of Niacin and Folic Acid in the Rat. *Am. J. Physiol.*, 148: 91-97. 1947.
- (96) TRAUBE, M. Ueber den Milchzucker als Medicament. *Deut. med. Wochschr.*, 7: 113-114. 1881.
- (97) VERZAR, F., AND MCDUGALL, E. J. Absorption from the Intestine. Longmans, Green, and Co., London, New York, Toronto. 1936.
- (98) WEINSTEIN, L., WEISS, J. E., AND GILLESPIE, R. W. H. The Influence of Diet on the *L. acidophilus* Content and the H-ion Concentration of the Intestine. *J. Bact.*, 35: 515-525. 1938.
- (99) WHITTIER, E. O. Lactose: A Review. *Chem. Revs.*, 2: 85-125. 1925.
- (100) WHITTIER, E. O. Lactose and its Utilization: A Review. *J. Dairy Sci.*, 27: 505-537. 1944.
- (101) WHITTIER, E. O., CARY, C. A., AND ELLIS, N. R. The Effects of Lactose on Growth and Longevity. *J. Nutrition*, 9: 521-532. 1935.

ASCORBIC ACID OXIDATION IN MILK BY PREFORMED HYDROGEN PEROXIDE

VLADIMIR N. KRUKOVSKY

*Department of Dairy Industry
Cornell University, Ithaca, N. Y.*

The following experiments were undertaken to ascertain whether the activity of peroxidase or of some other substances were responsible for the rapid promotion of oxidation of ascorbic acid in milk to dehydroascorbic acid by preformed H_2O_2 . The preliminary information pertaining to the subject was obtained in connection with the studies of the mechanism of the reactions involving ascorbic acid oxidation by added H_2O_2 , resulting in the stabilization of the lipid component of the milk system (4, 5). It was observed that the rate of ascorbic acid oxidation varied inversely with the volume of 30 per cent H_2O_2 added to milk, and the reaction was retarded appreciably when the amount of the reagent added was in excess of that required to oxidize ascorbic acid completely (5). From 0.021 to 0.03 ml. of 30 per cent H_2O_2 usually was needed to complete ascorbic acid oxidation in the milk. This retardation of ascorbic acid oxidation suggested that the reaction is catalyzed by an enzyme which in turn is slowly inactivated by the H_2O_2 . It was of interest, therefore, to learn if the heat inactivation of peroxidase in milk would result in non-reactivity of ascorbic acid and H_2O_2 , and if the ability of milk to utilize H_2O_2 for the oxidation of ascorbic acid could be restored by the addition of plant peroxidase.

The experimental conditions adopted for this study also were found to be convenient for the reinvestigation of the copper catalysis of ascorbic acid oxidation by H_2O_2 . This approach was prompted by the studies of Hand and Chase (3), which indicated that the differences between copper and light in their effects on the oxygen combining power of vitamin C could be accounted for by the assumption that copper catalyzes the oxidation of ascorbic acid by H_2O_2 .

EXPERIMENTAL

Zilva (8), in his work on the rate of heat inactivation of peroxidase in milk, has shown that heating for several hours at 65° C. produced no effect on the peroxidase activity of milk, and that 1.3 minutes at 75° C. reduced its activity to 10 per cent. Consequently, the samples of milk used in the present study were heated for 30 minutes at 61.1 and 76.6° C., respectively. Portions of these samples then were treated with either one or several reagents in the following order (per liter of milk): 0.09 ml. of H_2O_2 (10 per cent, standardized by titration with potassium permanganate), 1 ml. of horseradish peroxidase solution (1) and 0.1 mg. of copper (as copper sulphate). They were held at 20° C. during the first hour of digestion and then at 2 to 5° C. throughout the duration of the experiment. The changes in the ascorbic acid content of

Received for publication August 27, 1948.

the samples were followed by direct titration with 2,6-dichlorophenolindophenol in acid solution (6).

A comparison of data presented in figure 1 revealed that ascorbic acid was oxidized rapidly and completely when H_2O_2 was added to milk heated to 61.1°C ., and that the reagent was not utilized for the oxidation of ascorbic acid in the milk heated to 76.6°C ., the temperature at which, according to Zilva (8), the inactivation of peroxidase should be rapid and complete. The reaction was induced again, however, by the addition of horseradish peroxidase solution to non-reactive milk.

These results definitely indicate that peroxidase in milk plays an important

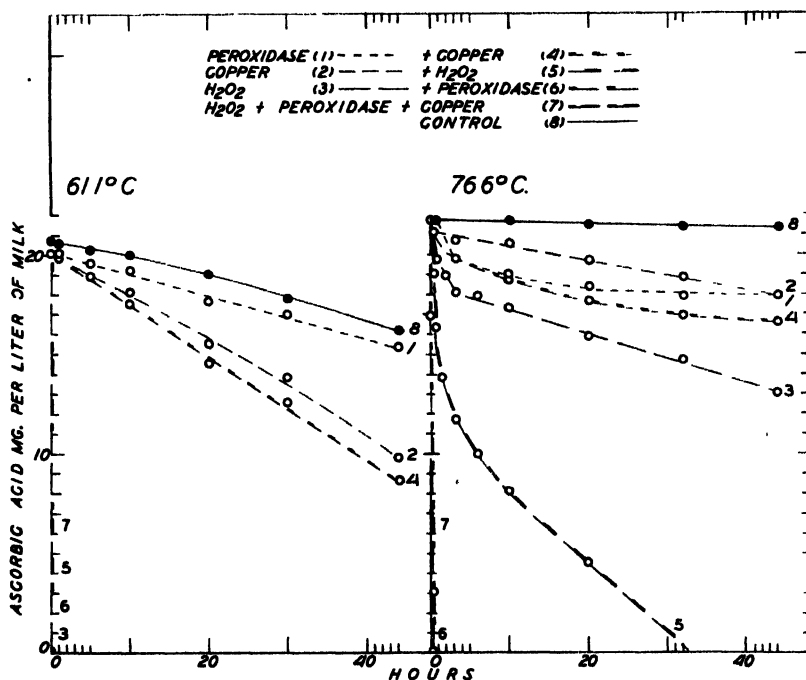


FIG. 1. The effects of the heat treatment of milk and of the subsequently added horseradish peroxidase and copper upon the rate of ascorbic acid oxidation by added hydrogen peroxide.

part in the reaction involving ascorbic acid oxidation in the presence of added H_2O_2 . It does not imply, however, that the peroxidase in milk utilizes H_2O_2 directly for the oxidation of ascorbic acid. It is possible that the foregoing reaction proceeds in the milk via a series of reactions, resembling the system which was described by Tauber in his study of the interaction of ascorbic acid and peroxidase (7).

It also could be seen that only a part of the ascorbic acid in the milk heated to 76.6°C ., as indicated by the slope of the curve, was oxidized rapidly by H_2O_2 with copper as a catalyst. It has been explained recently that copper acts as

an accelerator of ascorbic acid oxidation to dehydroascorbic acid in milk by atmospheric oxygen and brings the system more quickly to the critical point at which the reaction is forced to deviate from its original course. This critical point was thought to be governed primarily by the ascorbic-dehydroascorbic acids equilibrium (5). This statement was supported by the observations showing that the reaction responsible for the breakdown of the lipid component of the milk system, reaction which manifests itself by a rapid development of the intense tallowy flavor, is initiated more readily when a certain pressure between these two forms of vitamin C has been established. Since, in the course of ascorbic acid oxidation by H_2O_2 with copper as a catalyst, dehydroascorbic acid, as determined by the Gunsalus and Hand method (2), accumulates in the system, it would be logical to assume that the establishment of an ascorbic-dehydroascorbic acid pressure caused the reaction to slow down. As before, it resulted in the promotion of the reaction which produces the off flavors in milk. This secondary reaction was not promoted when ascorbic acid was oxidized with hydrogen-peroxide-peroxidase, either natural or added.

In addition, it should be pointed out that when H_2O_2 was added to milk in excess of the amount needed to oxidize its ascorbic acid content (from 0.03 to 0.12 ml. of 30 per cent H_2O_2 per liter of milk), the subsequent heating for 30 minutes at 61.1°C . invariably resulted either in retardation or inhibition of ascorbic acid oxidation by H_2O_2 , as determined by the addition of these reagents to milk after the heat treatment.

CONCLUSIONS

Evidence is presented to show that peroxidase in milk might be responsible for the quick conversion of ascorbic acid to dehydroascorbic acid by added hydrogen peroxide. The data show that the reagent was not utilized for the oxidation of ascorbic acid in the milk heated to 76.6°C ., and that the reaction could be induced again by the addition of plant peroxidase.

REFERENCES

- (1) GJESSING, E. C., AND SUMNER, J. B. Synthetic Peroxidases. *Arch. Biochem.* 1: 1-8: 1943.
- (2) GUNSALUS, I. C., AND HAND, D. B. The Use of Bacteria in the Chemical Determination of Total Vitamin C. *J. Biol. Chem.*, 141: 853-858. 1941.
- (3) HAND, D. B., AND GREISEN, E. C. Oxidation and Reduction of Vitamin C. *J. Chem. Soc.*, 64: 358-361. 1942.
- (4) KRUKOVSKY, V. N., AND GUTHRIE, E. S. Ascorbic Acid Oxidation, a Key Factor in the Promotion of the Tallowy Flavor in Milk. *J. Dairy Sci.*, 28: 565-579. 1945.
- (5) KRUKOVSKY, V. N., AND GUTHRIE, E. S. Vitamin C, Hydrogen Peroxide, Copper and the Tallowy Flavor in Milk. *J. Dairy Sci.*, 29: 293-306. 1946.
- (6) SHARP, P. F. Rapid Method for Quantitative Determination of Reduced Ascorbic Acid in Milk. *J. Dairy Sci.*, 21: 85-88. 1938.
- (7) TAUBER, H. The Interaction of Peroxidase and Ascorbic Acid in Biological Oxidations and Reductions. *Enzymologia*, 1: 209-212. 1936.
- (8) ZILVA, S. S. The Rate of Inactivation by Heat of Peroxidase in Milk. *Biochem. J.*, 8: 656-669. 1914.

PRESERVATION OF DAIRY PRODUCTS FOR THE PHOSPHATASE TEST

GEORGE P. SANDERS AND OSCAR S. SAGER

*Division of Dairy Products Research Laboratories, Bureau of Dairy Industry, Agricultural
Research Administration, U. S. Department of Agriculture, Washington 25, D. C.*

Results of recent investigations have shown that the alkaline phosphatase of cow's milk is very stable in dairy products that have not undergone spoilage. For example, it was shown (7) that the enzyme retains a large proportion of its activity, sufficient for determination as an index of pasteurization, in Cheddar cheese which had been held in storage for several years. High activity likewise has been found, after storage for many months under usual refrigeration conditions, in samples of soft and semi-soft cheeses, butter, ice cream and sherbet made from under-pasteurized milk or cream. Scharer (10) showed that the enzyme in milk retains nearly all of its activity under storage conditions for considerable periods, in some instances as long as 7 to more than 10 days. Results of research in the authors' laboratories have demonstrated also that fluid dairy products and perishable soft cheeses, prepared for consumption while fresh, retain their phosphatase activity for a much longer time if stored under refrigeration conditions adequate to effectively retard spoilage than if kept at room temperature.

However, when spoilage takes place, considerable loss of phosphatase activity occurs. Scharer (10) pointed out that this loss may be due to the increase in acidity coupled with the inadequacy of the buffer under such extreme conditions to maintain the optimum pH for activity of the enzyme. Scharer also pointed out that it is difficult to obtain a representative sample of a milk that has spoiled. Results here have verified the hypothesis that excessive acidity causes partial inactivation of phosphatase. Furthermore, the results have demonstrated also that the increase in acidity makes it necessary, for reliable results, to modify the test by increasing the concentration and the alkalinity of the buffer in order to produce a pH of approximately 10, which is optimum for enzymic hydrolysis (8) (9).

In addition to the partial inactivation of the enzyme that results from a marked decrease in pH, our results have shown that additional inactivation occurs in instances in which souring and spoilage are accompanied by proteolysis. This inactivation suggests a decomposition of the base protein of the phosphatase by proteolytic enzymes produced by microorganisms. Like the inactivation produced by heat, this loss of activity accompanying proteolysis cannot be restored.

Conversely, in some instances sufficient microbial phosphatase may be produced by abundant growth of certain microorganisms during spoilage of dairy products to yield so-called "false positive" tests, thus seriously complicating the results and the interpretation thereof.

Received for publication August 27, 1948.

Hahn and Tracy (2) investigated the use of mercuric chloride, sodium salicylate, sodium tetraborate (borax), hydrogen peroxide and formaldehyde for preserving fluid milk to be tested for phosphatase. They kept samples at room temperature for 3 days and used the Scharer laboratory method for testing. They concluded that mercuric chloride was the only one of these chemicals which was satisfactory. The concentration of mercuric chloride used was stated to be one tablet in one-half pint of milk. Newlander (6) described two sizes of tablets, one containing 0.225 g. and another 0.45 g. of the chloride. Thus, the concentration of mercuric chloride used in milk by Hahn and Tracy may have been either 0.09 or 0.18 per cent.

Neave (5) found that mercuric chloride in a concentration of 0.01 per cent "increased the keeping quality of the milk from 1 to 8 days at 68° F." and had no significant effect on milk phosphatase in samples tested by a modification of the Kay-Graham method. However, a concentration of 0.02 per cent, which apparently is much less than that used by Hahn and Tracy, had some destructive effect on the enzyme. Later, Scharer (11) reported that one tablet of mercuric chloride in one-half pint of milk completely destroyed the enzymic activity in 1 day in pasteurized milk with 0.5 per cent of raw milk added. His results indicated that 2 g. of borax per one-half pint of milk, a concentration of approximately 0.8 per cent, decreased the phosphatase activity in the test from 5 to 3.5+ units in 3 to 5 days, but that it was the only effective common preservative.

Barber and Frazier (1) stated that mercuric chloride does not interfere with the test. They used 3 per cent of it to inhibit bacterial growth in pasteurized cream containing phosphatase-producing bacteria, and the results of phosphatase tests after storage were negative.

Mucciolo and Cerveira (4) reported that a concentration of 0.005 per cent of formaldehyde had no appreciable effect on the enzyme in milk stored at room temperature for 72 hours. However, the accuracy of their results is not conclusive, since the phenol values stated to be unaffected by the preservative were reported as "more than 5 Scharer units," a value much too low to indicate quantitatively the actual phosphatase activity of raw milk.

The use of chloroform was recommended by Julien *et al.* (3) as a result of a study of various chemicals for the preservation of samples of cheese to be tested for extraneous matter.

Because quantitative methods for precisely measuring phosphatase activity in dairy products were not available, it was not possible until recently to secure accurate information concerning the kinds, concentrations and inhibitory effects of the chemicals that might be used to preserve dairy products for the test. The quantitative phosphatase test developed in these laboratories (8) requires the use of some reagents that are different from those used in earlier tests and involves different and more precise control of the pH, as was pointed out earlier (9). In view of these modifications, it was conceivable that the newer test might give different and more conclusive results.

In view of the need for increasing the usefulness and the application of the test for all dairy products as an index of pasteurization, and because of the importance of preserving samples that require shipment without refrigeration to a laboratory for testing, it was desirable to investigate the use of preservatives with the Sanders-Sager test (8). The objectives of this research were to determine the kinds and concentrations of various chemicals that would preserve milk and some other milk products for various periods of time at room temperature; the possible inhibitory effects of various preservatives on the activity of the enzyme; and the possible interfering effects of preservatives in the test.

METHODS

The milks used were fresh and of high quality and were taken from the composite milks obtained from a large herd. All samples were prepared by pasteurizing a large part of each mixed lot and then adding raw milk in a proportion of 2.5 per cent. This was done principally to reduce the enzymic activity to a range in which the results of tests could be determined without making dilutions. Thus, the enzymic activities as well as the effects of preservatives were comparable with those that would be found in grossly under-pasteurized milk and in pasteurized milk contaminated with considerable raw milk.

Seven lots of milk were used, and 15 to 18 samples, with various preservatives in different quantities, were prepared from each lot. Thus, more than 110 samples were tested during storage. The flasks and stoppers were sterilized, 100 ml. of milk put in each flask, the preservative added and the contents mixed. The pH values were determined and phosphatase tests were begun within an hour after the addition of preservative. Subsequent pH and phosphatase determinations were made after storage at room temperature, which was between 25 and 29° C., for 1, 3, 5, 7, 10, 14 and 21 days, or until extreme spoilage occurred. A sample of milk from each lot was stored at 3 to 5° C. as an untreated, refrigerated control and was tested at similar intervals. The samples were examined daily for physical evidences of spoilage, including lumping of the fat, separation of whey, formation of gas, curdling and unclean odors.

In some instances in which the phosphatase values increased during storage, *i.e.* in a few samples containing borate, tests for microbial phosphatase were conducted by heating 1 ml. of the product in a tube for 5 minutes at 70° C. to destroy the milk enzyme, cooling to room temperature and then conducting the test in the usual way. A positive test on a sample pasteurized thus is a reliable indication of the presence of microbial phosphatase, *i.e.* of a so-called false positive result. Microbial-phosphatase controls were prepared by adding 1 ml. of the appropriate barium borate-hydroxide buffer (pH 10.6) to 1 ml. of milk and heating the mixture (pH approximately 9.5) in boiling water for 5 minutes to destroy microbial phosphatase, cooling and conducting the test.

The samples of cheese were plugs of Cheddar and Swiss cheese, weighing approximately 10 g., taken with a trier. They were placed in 1- by 8-inch test tubes, containing plugs of cotton moistened with chloroform, as described by

Julien *et al.* (3). Various quantities of chloroform, from 0.5 to 3 per cent of the volume of the containers, were used. The tubes with samples were stoppered tightly and kept at room temperature. Similar samples, without preservative, were stored at 3 to 5° C. as controls.

Determinations of the pH values of fluid samples were made with a Beckman glass-electrode pH meter. Phosphatase tests were made by the Sanders-Sager method (8).

RESULTS

The preservatives that were tested experimentally in milk at room temperature are listed below, with the respective concentrations used and the numbers of days that elapsed before spoilage occurred, as indicated by physical

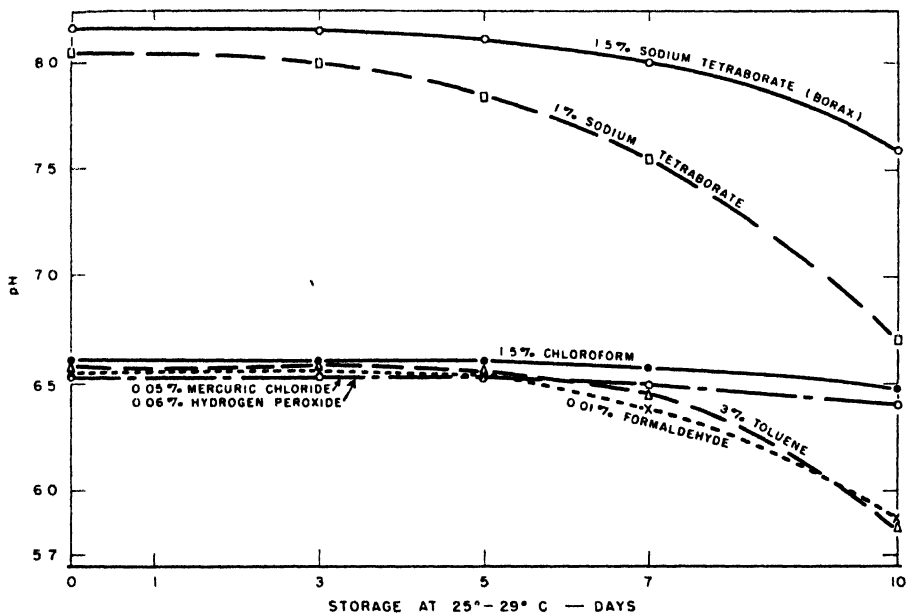


FIG. 1. Minimal concentrations of preservatives required to preserve milk for 7 to 10 days (2.5 per cent of raw milk in pasteurized milk).

changes, principally curdling, and by a relatively steep slope of the pH curve to approximately 5 or slightly lower.

Chloroform: 0.25 per cent, 2 days; 0.5 per cent, 2-3 days; 1 per cent, 6-10 days; 1.5 per cent, 10-21 days; 2 per cent, > 21 days.

Toluene: 1 per cent, 2 days; 2 per cent, 6 days; 3 per cent, 8-12 days; 3.5 per cent, > 21 days.

Sodium tetraborate (borax): 0.25 per cent, 3-4 days; 0.5 per cent, 4-7 days; 0.75 per cent, 8-10 days; 1 per cent, 8-14 days; 1.5 per cent, 10-18 days; 2 per cent, 14- > 21 days.

Sodium metaborate: 0.25 per cent, 4 days; 0.5 per cent, 7 days; 0.75 per cent, 11 days; 1 per cent, 13 days.

Sodium tetraborate-sodium metaborate mixture, equal parts by weight: 0.25 per cent, 3-4 days; 0.5 per cent, 4-6 days; 0.75 per cent, 7-10 days; 1 per cent, 8 days; 1.5 per cent, 10 days; 2 per cent, 13 days.

Formaldehyde: 0.005 per cent (*i.e.*, 0.0125 per cent of a 40 per cent solution by volume), 4-7 days; 0.01 per cent, 6-14 days; 0.03 per cent, 14-21 days; 0.05 per cent, 16 days; 0.1 per cent, > 21 days.

Mercuric chloride: 0.005 per cent, 2 days; 0.01 per cent, 3-6 days; 0.025 per cent, 6-7 days; 0.05 per cent, 7-14 days; 0.1 per cent, > 21 days.

Hydrogen peroxide: 0.015 per cent (*i.e.*, 0.05 per cent of a 30 per cent solution), 2 days; 0.03 per cent, 3-4 days; 0.06 per cent, 8-16 days; 0.09 per cent, 10-21 days; 0.15 per cent, > 21 days.

Correlation of the pH data with the results of physical examinations of the samples showed that changes in pH generally were a reliable criterion of the

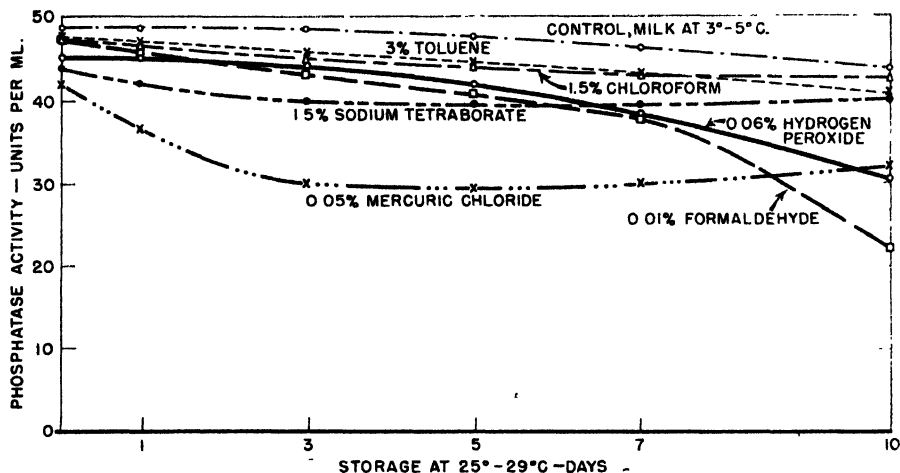


FIG. 2. Effects of preservatives on phosphatase activity of milk in storage for 10 days (2.5 per cent of raw milk in pasteurized milk).

physical changes indicative of spoilage. The effectiveness of the chemicals for preserving milk was evaluated, therefore, on the basis of changes in pH. Illustrative data showing the minimal concentration of each chemical that was effective in preserving the milk adequately for testing, without any marked, undesirable changes in physical characteristics, for periods of 7 to 10 days are presented in figure 1, and for periods of 14 to 21 days in figure 3.

The results of phosphatase tests on these samples, showing the inhibitory effects on the phosphatase activity produced by the concentrations of chemicals indicated in figures 1 and 3, are presented in figures 2 and 4, respectively.

The phosphatase values of the controls stored at 3 to 5° C. without preservative, also shown in figures 2 and 4, decreased in 10 days from an average of 48.5 to 43.5 units per ml., a loss of 10 per cent; and during the same period the pH values decreased from an average of 6.60 to 6.30. During 21 days the

average loss in enzymic activity in the controls was 20 per cent, and the pH decreased to an average of 5.20. These controls generally curdled during the third week.

All of the chemicals tested, when used in suitable concentrations, were effective in preserving the samples of milk for the periods of time indicated. However, they differed greatly in the extent to which they inhibited the enzyme. All of the chemicals tested, except chloroform and toluene, produced a considerable decrease in phosphatase activity. The increasing order of their destructive effects was as follows: chloroform, toluene, sodium tetraborate (borax), formaldehyde, mercuric chloride and hydrogen peroxide.

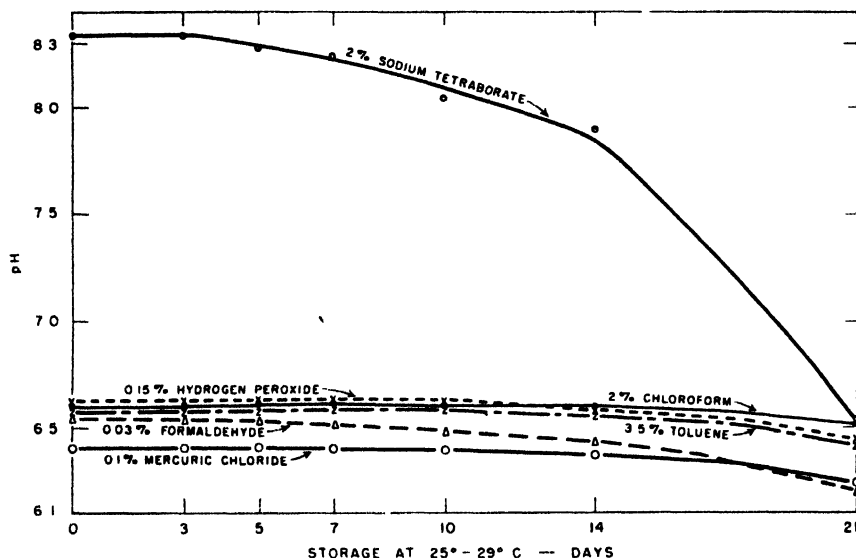


FIG. 3. Minimal concentrations of preservatives required to preserve milk for 14 to 21 days (2.5 per cent of raw milk in pasteurized milk).

Only chloroform and toluene had no seriously inhibitory effects on the enzymic activity. The use of 1.5 to 2 per cent of chloroform or 3 to 3.5 per cent of toluene at 25 to 29° C. generally produced no more or very little more inactivation than was incurred under refrigeration at 3 to 5° C. Chloroform is considered to be more suitable than toluene as a preservative, because it is effective in lower concentration and also because it effectively prevents lumping of the fat for an indefinite period, especially when used in a concentration of 1.5 per cent or greater. Moreover, both have distinctive odors, thus reducing greatly the danger of accidental poisoning.

Sodium tetraborate, in concentrations sufficient to preserve milk, not only inhibited the enzyme more than did either chloroform or toluene (fig. 2 and 4), but also interfered in the test because of its marked buffering effect. When it was present, the pH values in the test generally were about 9.7 instead of within the optimal range of 9.85 to 10.2 (8, 9). Thus, it was necessary to modify the

test by using a more concentrated barium borate buffer of greater alkalinity. Attempts to produce optimal pH conditions in the test by substituting sodium metaborate, which buffers at a higher pH value, were not successful. Moreover, the metaborate was not found to be superior to the tetraborate as a preservative.

Increases in phosphatase values (not included in figures), beginning generally at 10 to 14 days, were found in occasional instances in only the samples containing borates, and tests made of portions of these samples following pasteurization at 70° C. for 5 minutes showed that the increases were due to microbial phosphatase. This condition may have resulted from the activity of phosphatase-producing organisms whose growth may be favored by the relatively high pH produced in milk by borate (figs. 1 and 3).

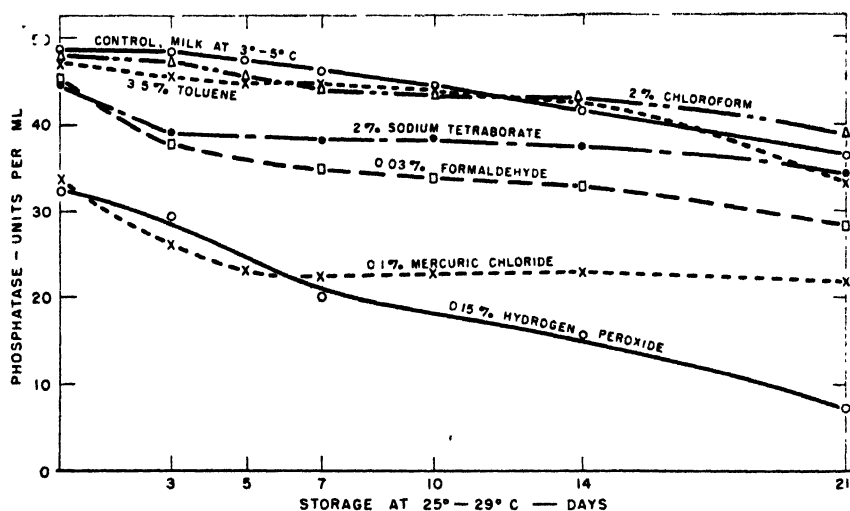


FIG. 4. Effects of preservatives on phosphatase activity of milk in storage for 21 days (2.5 per cent of raw milk in pasteurized milk).

Figures 2 and 4 also show that the enzymic activity was inhibited appreciably by 0.01 to 0.03 per cent of formaldehyde and more markedly by 0.05 to 0.1 per cent of mercuric chloride and also by 0.06 to 0.15 per cent of hydrogen peroxide. The results of the tests begun within an hour after the addition of preservatives show that the inhibitory effects largely are immediate, regardless of storage, and are attributable to chemical effects of the preservatives upon the enzymic activity.

Considerable lumping of the fat prior to curdling occurred with peroxide, mercuric chloride and formaldehyde, and some lumping occurred with tetraborate and toluene, making it relatively difficult to secure homogeneous samples after storage. The use of chloroform eliminated this difficulty.

Samples of hard cheese, stored at room temperature in stoppered tubes containing chloroform, showed no evidence of growth of mold within 3 weeks with

a quantity of chloroform equal to 0.75 per cent or more of the volume of the container, and none within 1 month with 1 per cent or more. The largest decrease in phosphatase activity in 1 month at room temperature in those in which 1 per cent of chloroform had been added was 8 per cent, and the largest decrease in 1 month with 1.5 per cent was 6 per cent. No microbial phosphatase could be detected in tests made on samples taken from the surfaces of the preserved cheese.

Some development of mold was found on the control samples of cheese stored at 3 to 5° C. without chloroform, beginning generally during the late part of the third week on the Cheddar samples and somewhat later on the Swiss samples. No evidence of microbial phosphatase could be detected in or on the surfaces of the refrigerated samples prior to 1 month.

The results with chloroform conformed with those of Julien *et al.* (3) to the effect that it is an ideal preservative.

It was pointed out by Scharer (11) that salicylates are not suitable for preserving samples to be tested for phosphatase, because salicylates react with 2,6-dibromoquinonechloroimine (BQC), producing the blue color of indophenol. This conclusion was verified in our experiments with sodium salicylate. Likewise, cresols, resorcinols and naphthols were found unsuitable, because they also yield interfering colors.

Without exception, samples to which a preservative has been added should be labeled plainly in the following or a similar manner: "*Poison, preservative added.*"

It should be recognized that concentrations of preservatives greater than those described herein may be necessary to preserve entirely raw milk for comparable periods of time under summer conditions.

SUMMARY AND CONCLUSIONS

Research on the preservation of milk for the phosphatase test showed that each of the chemicals listed below will preserve fresh milk (in these experiments, mixtures of 2.5 per cent of fresh, raw milk in fresh, pasteurized milk) for periods as long as 10 days to 3 weeks under summer conditions at room temperature. The preservatives tested, arranged in increasing order of their inhibitory effects on milk phosphatase, were as follows: 1.5 to 2 per cent of chloroform, 3 to 3.5 per cent of toluene, 1.5 to 2 per cent of sodium tetraborate (borax), 0.01 to 0.03 per cent of formaldehyde (0.025 to 0.075 per cent of a 40 per cent solution by volume), 0.05 to 0.1 per cent of mercuric chloride, and 0.06 to 0.15 per cent of hydrogen peroxide (0.2 to 0.5 per cent of a 30 per cent solution).

Chloroform was more effective than toluene as a preservative in the same concentration. Neither inhibited the enzyme appreciably. Other advantages of chloroform are that it prevents the fat emulsion from breaking, thus eliminating the separation of lumps of fat on the surface, and its presence can be detected very easily because of its distinctive odor, thus reducing greatly the danger of accidental poisoning.

Hydrogen peroxide, mercuric chloride and formaldehyde produced serious chemical inhibition of the activity of milk phosphatase. Sodium tetraborate had only a slight inhibitory effect, but the quantity of it necessary for preservation yielded in milk pH values of approximately 9.7 in the phosphatase test instead of optimal pH values of 9.85 to 10.2, and thus it interfered in the test. This excessive buffering interference could not be corrected adequately by substituting the more alkaline sodium metaborate as a preservative.

Phenolic compounds, including salicylates, cresols, resorcinols and naphthols, should not be used as preservatives in connection with the test, because they react with BQC and yield blue or other interfering colors.

It is recommended that 1.5 to 2 per cent of chloroform be used to preserve fluid dairy products for the phosphatase test. For solid samples, including cheese, it is recommended that chloroform, in a quantity equal to 1.5 to 2 per cent of the volume of the container, be put on a wad of cotton in the sample tube or jar with the sample and that the container be stoppered tightly.

REFERENCES

- (1) BARBER, F. W., AND FRAZIER, W. C. The Development of a Positive Phosphatase Test in Refrigerated, Pasteurized Cream. *J. Dairy Sci.*, 26: 343-352. 1943.
- (2) HAHN, A. J., AND TRACY, P. H. Controlling Milk Pasteurization Efficiency through the Use of the Phosphatase Test. *J. Dairy Sci.*, 22: 191-200. 1939.
- (3) JULIEN, J. P., DUMAIS, R., AND THIBODEAU, R. Control of Extrinsic Matter in Cheddar Cheese. *Can. Dairy and Ice Cream J.*, 26, 10: 34, 36. 1947.
- (4) MUCCIOLO, P., AND CERVEIRA, M. Influence of Formaldehyde as a Milk Preservative on the Peroxidase, Reductase, and Phosphatase Tests. *Rev. faculdade med. vet., Univ. São Paulo*, 3: 127-129. 1946.
- (5) NEAVE, F. K. Kay and Graham's Phosphatase Test. *J. Dairy Research*, 10: 475-484. 1939.
- (6) NEWLANDER, J. A. The Testing and Chemistry of Dairy Products. Olsen Pub. Co., Milwaukee, Wis. p. 48. 1946.
- (7) SANDERS, G. P., AND SAGER, O. S. Development of a Phosphatase Test Applicable to Cheddar Cheese. *J. Assoc. Offic. Agr. Chemists*, 28: 656-675. 1945.
- (8) SANDERS, G. P., AND SAGER, O. S. Phosphatase Test for Various Dairy Products. *J. Dairy Sci.*, 30: 909-920. 1947.
- (9) SANDERS, G. P., AND SAGER, O. S. Present Status of the Phosphatase Test. *J. Milk Technol.*, 11: 67-75. 1948; and *Cherry-Burrell Circle*, Pp. 3-5, 25-26. Mar.-Apr., 1948.
- (10) SCHAREE, H. A Rapid Phosphomonoesterase Test for Control of Dairy Pasteurization. *J. Dairy Sci.*, 21: 21-34. 1938.
- (11) SCHAREE, H. Further Observations on the Rapid Phosphatase Test. *Am. J. Pub. Health*, 30: 1206-1210. 1940.

FROZEN HOMOGENIZED MILK VI. THE USE OF STABILIZERS IN FROZEN HOMOGENIZED MILK

C. J. BABCOCK,¹ CAPTAIN JOSEPH N. STABILE, MSC, USA, ERNEST S. WINDHAM,
AND COLONEL RAYMOND RANDALL, VC, USA

Veterinary Division, Army Medical Center, Washington 12, D. C.

According to Tressler and Evers (10), the freezing of whole milk and condensed milk is not being practiced on any large commercial scale, but since there is a growing demand for this product the industry should develop into a sizable one. The use of frozen homogenized milk to supply fresh milk to patients on hospital ships during World War II (1) has caused considerable interest in this product. However, there are two major problems which must be overcome before homogenized milk can be held for long periods of time in the frozen state before use. They are the development of methods for the preservation of flavor quality and the prevention of separation of the milk solids which is evident, at times, on thawing.

While the use of stabilizers in ice cream and evaporated milk is widespread, reports of their use in frozen homogenized milk have not been found. However, Seekles and Smeets (9) report that the stabilization of milk without altering its pH may be affected by adding certain substances which cause the activity of the calcium ions to decrease. These workers found that sodium citrate slightly decreased the rate of flocculation, and that calcium-sequestering polyphosphates definitely retarded it but introduced a readily detectable astringent flavor. The desirability of delaying the appearance of protein flocculation and flavor defects is strongly indicated in reports of previous investigations (1, 2, 3, 4, 5, 6, 8, 11, 12).

EXPERIMENTAL

The present experiments were undertaken to determine the ability of certain chemicals to act as stabilizers and thereby materially increase the storage life of frozen homogenized milk. Homogenized milk with a fat content of 3.8 per cent, pasteurized by holding at 155° F. for 30 minutes and packaged in one-half pint paper containers by a commercial dairy was used. With the exception of ascorbic acid, the quantity of each stabilizer required to impart a detectable flavor to the milk was determined for the first series. A slightly smaller quantity then was added to the milk to be frozen. This amount of hydrogen peroxide used as a stabilizer, however, caused the milk to have an astringent flavor after it had been frozen and thawed and failed to reduce materially the bacterial content of the milk either before or after freezing.

The chemicals used and their concentration per liter of homogenized milk were as follows: (a) ascorbic acid, 0.1 g.; (b) pectin, 1 g.; (c) calcium chloride, 0.5 g.; (d) urea, 3 g.; (e) carboxyl methyl cellulose, 0.5 g.; (f) sodium citrate, 2 g.; (g)

Received for publication August 30, 1948.

¹ Formerly Lieutenant Colonel, SnC, AUS. Present address, Dairy Branch, Production Marketing Administration, U. S. Department of Agriculture.

TABLE 1
Influence of various chemicals on the stability of frozen homogenized milk stored at -17.8°C . (0°F .)

Storage time	Control	Chemical added					Hydrogen peroxide
		Ascorbic acid	Pectin	Calcium chloride	Urea	Carboxyl methyl cellulose	
Degree of separation (ML sediment/50 ml. milk)							
(days)							
5	0.01	0.01	0.01	0.01	0.01	0.01	0.03
15	0.01	0.01	0.01	0.01	0.01	0.01	0.03
27	0.02	0.01	0.02	0.02	0.02	0.02	0.02
35	0.02	0.01	0.03	0.02	0.02	0.02	0.02
44	0.03	0.02	0.02	0.02	0.02	0.02	0.02
55	0.03	0.03	0.10	0.02	0.20	0.70	0.02
65	1.50	0.90	0.20	0.60	0.20	3.50	2.00
75	0.40	0.40	0.70	1.80	0.20	1.10	3.00
85	0.90	0.90	1.20	2.00	0.20	2.00	5.00
95	3.00	0.90	1.50	2.80	0.30	4.50	10.00
105		1.20	5.00	6.00	0.50	10.00	6.50
115		1.80	2.20	2.20	0.40	7.50	11.00
125		1.60	1.20	6.00	0.50	13.50	12.00
135		5.50	6.50	9.00	1.20	11.00	14.00
145		12.00	13.00		2.50	14.00	15.00
155							
175							
Flavor							
5	Normal	Normal	Normal	Normal	*Sl. flat	Sl. abnormal	Sl. flat
15	Normal	Normal	Normal	Sl. salty	Sl. flat	Normal	Sl. flat
27	Normal	Normal	Normal	Sl. salty	Sl. flat	Normal	Sl. flat
35	Normal	Normal	Normal	Normal	Sl. flat	Normal	Normal
44	Normal	Normal	Normal	Normal	Sl. flat	*Tr. oxidized	Normal
55	Normal	Normal	Normal	Normal	Sl. flat	Normal	Sl. flat
65	*V. sl. old	Normal	Normal	Normal	Sl. flat	Sl. oxidized	Sl. old
75	Sl. oxidized	Normal	Sl. oxidized	Sl. oxidized	V. sl. old	Sl. oxidized	Old
85	V. sl. old	Normal	Oxidized	V. sl. oxidized	V. sl. old	Sl. oxidized	Old
95	V. sl. old	Normal	Oxidized	Sl. oxidized	*Tr. oxidized	Oxidized	Normal
105	Normal	Oxidized	Sl. oxidized	Tr. oxidized	Oxidized	Lacks freshness
115	..	V. sl. old	Oxidized	Oxidized	Oxidized	St. oxidized	Abnormal
125		Sl. old	Oxidized	St. oxidized	Oxidized	St. oxidized	Abnormal
135		Sl. old	Oxidized	St. oxidized	Oxidized	St. oxidized	Abnormal
145		V. sl. oxidized	*St. oxidized	St. oxidized	Oxidized	St. oxidized	Sl. oxidized
155							Oxidized
175						Old-chalky	Old
							*Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent
							Ab. astringent

*Sl. = slightly; *V. sl. = very slightly; *St. = strongly; *Tr. = trace; *Ab. = Abnormal

disodium phosphate, 1 g.; and (h) 30 per cent hydrogen peroxide, 1 ml. An additional group of samples to which no chemical was added served as a control group.

The samples were frozen and stored at about $-17.8^{\circ}\text{C}.$ ² They were removed and thawed for examination at intervals of about 10 days beginning with the fifth storage day. The degree of separation of the milk was measured by determining the quantity of sediment in 50 ml. by centrifuging, as was done in previously reported experiments (1). Flavor determinations again were made by a panel of three men experienced in milk judging.

Table 1 shows that sodium citrate was of greater value as a stabilizer than any of the other substances used. Separation was not noticeable in the milk samples containing sodium citrate until they had been stored for 145 days, and flavor deterioration was not evident until at least 105 days. Noticeable separation had taken place and flavor deterioration had become evident in the control samples after a storage period of 65 days. The milk with added ascorbic acid showed separation at the same storage age as did the control samples, but its addition preserved the flavor slightly longer than did the addition of sodium citrate. The milk samples containing pectin, calcium chloride, carboxyl methyl cellulose and disodium phosphate each showed separation and flavor deterioration at about the same time as the control group. Urea and hydrogen peroxide each delayed the occurrence of separation but did not help to preserve the flavor of the milk.

To further establish their value as stabilizers in frozen homogenized milk, sodium citrate and sodium citrate in combination with ascorbic acid were used under different storage conditions. The homogenized milk used was of the same quality and was packaged in the same manner as that used in the first series. The three equal groups consisted of a control group, a group to which 2 g. of sodium citrate per liter was added and a group to which, in addition to the sodium citrate, 100 mg. per liter of ascorbic acid was added.

It has been shown that if homogenized milk is frozen and held for short periods of time at a low temperature and then moved and held in a frozen state at a much higher temperature, the stability of the milk is affected adversely (1). To simulate this adverse condition of storage, each of the three main groups was divided into sub-groups, first frozen and then treated as follows: A_1 held at a constant temperature of $-11.5^{\circ}\text{C}.$; A_2 held at $-17.5^{\circ}\text{C}.$; B_1 held at $-27.5^{\circ}\text{C}.$ and after 5 days held at $-17.5^{\circ}\text{C}.$; B_2 held at $-27.5^{\circ}\text{C}.$ and after 25 days held at $-17.5^{\circ}\text{C}.$; C_1 held at $-27.5^{\circ}\text{C}.$ and after 5 days held at $-11.5^{\circ}\text{C}.$; C_2 held at $-27.5^{\circ}\text{C}.$ and after 25 days held at $-11.5^{\circ}\text{C}.$; D_1 held at $-17.5^{\circ}\text{C}.$ and after 5 days held at $-11.5^{\circ}\text{C}.$; D_2 held at $-17.5^{\circ}\text{C}.$ and after 25 days held at $-11.5^{\circ}\text{C}.$ Representative samples of all sub-groups were examined at intervals of about 10 days for flavor and sediment.

Section A of table 2 shows that the addition of sodium citrate with ascorbic acid doubled the time that homogenized milk remained normal in appearance and flavor when frozen and stored either at $-11.5^{\circ}\text{C}.$ or at about $-17.8^{\circ}\text{C}.$ when compared with frozen milk without the addition of stabilizers. As was shown in table

² The thermostat controlling the temperature of the freezer had sufficient lag to cause a maximum temperature variation of about $8^{\circ}\text{C}.$

TABLE 2
*Influence of sodium citrate and sodium citrate with ascorbic acid on
 the stability of frozen homogenized milk*

A—Frozen and held at:						
(A ₁) -11.5° C.			(A ₂) -17.5° C.			
Total storage time	Character of sample					
	Control	Sodium citrate	Sodium Citrate with ascorbic acid	Control	Sodium citrate	Sodium Citrate with ascorbic acid
	Sediment in 50 ml. milk					
(days)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)
47	0.50	0.04	0.02	0.04	0.02	0.01
55	0.70	0.20	0.15	0.50	0.02	0.02
64	0.90	0.20	0.10	0.60	0.02	0.01
74	0.70	0.30	0.25	1.00	0.02	0.02
84	1.40	0.50	0.40	2.40	0.05	0.03
95	2.50	0.30	0.70	1.60	0.03	0.03
104	6.50	0.90	0.50		0.08	0.05
114		1.40	0.60		0.20	0.15
123	8.50	0.70	1.00	11.00	0.10	0.40
140	11.00	1.00	2.00	11.00	0.20	
154	10.50	2.00	1.10	12.00	0.80	0.70
Flavor						
47	Tr. oxidized ^a	Normal	Normal	Normal	Normal	Normal
55	Sl. oxidized ^b	Normal	Normal	Normal	Normal	Normal
64	Oxidized	Normal	Normal	Normal	Normal	Normal
74	Oxidized	Normal	Normal	Normal	Normal	Normal
84	Sl. oxidized	Sl. flat	Normal	Oxidized	Normal	Normal
95	Flat	Normal	Normal	Oxidized	Sl. flat	Normal
104	Sl. oxidized	Flat	Normal		Sl. flat	Normal
114	Oxidized	Flat	Normal		Sl. oxidized	Normal
123	Oxidized	Sl. oxidized	Sl. oxidized	Sl. oxidized	Sl. oxidized	Normal
140	..	Sl. oxidized	Sl. oxidized	Oxidized	Oxidized	
154	..	Oxidized	Oxidized		Oxidized	Old, v. sl. oxidized
B—Frozen and held at -27.5° C.; moved to -17.5° C.						
(B ₁) moved after 5 days			(B ₂) moved after 25 days			
Sediment						
25	0.02
35	0.02
47	0.10	0.01	0.02	0.10
55	0.30	0.02	0.02	0.05	0.02	0.02
64	0.50	0.01	0.01	0.30	0.02	0.02
74	1.00	0.02	0.02	0.50	0.02	0.02
84	..	0.03	0.05	1.00	0.03	0.07
95	..	0.04	0.03	3.50	0.04	0.03
104	..	0.07	0.05	0.03
123	0.02	..
140	0.02	..

Flavor						
25	V. sl. flat
35	Normal
47	V. sl. oxidized	Normal	Normal	Tr. oxidized
55	Sl. oxidized	Normal	Normal	Tr. oxidized	Normal	Normal
64	Sl. oxidized	Normal	Normal	Sl. oxidized	Normal	Normal
74	Sl. oxidized	Normal	Normal	Sl. oxidized
84	Sl. flat	Sl. oxidized	Sl. oxidized	Normal	Normal
95	Normal	Normal	Sl. oxidized	Normal	Normal
104	Sl. flat	Flat	Normal
123
140	Sl. oxidized

* Tr. = trace; ^b Sl. = slight; ^c V. sl. = very slight.

1, the addition of sodium citrate delays separation or flocculation in frozen homogenized milk stored at a constant temperature. When the milk was frozen and stored at -11.5°C ., noticeable separation had occurred in the control samples that were thawed after 55 days storage. This separation was not noticeable in the samples containing sodium citrate until they had been stored from 95 to 105 days. The control samples stored at about -17.8°C ., showed separation after storage for 64 days. The samples containing sodium citrate, when stored at this temperature, did not show separation for more than 140 days. At each of the storage temperatures used the addition of sodium citrate also delayed the development of abnormal flavors. The development of these flavors was delayed further by adding ascorbic acid to the samples containing sodium citrate. This finding was more pronounced in the samples stored at about -17.8°C . than it was in those stored at -11.5°C .

Sections B, C and D of table 2 show that the addition of sodium citrate and ascorbic acid prolonged the time that homogenized milk remained normal in appearance and flavor when it was frozen and held at one temperature and then stored at a higher temperature. When the homogenized milk was frozen and stored for 5 days at -27.5°C . and then moved to -17.8°C ., or frozen and stored for 5 days at -27.5°C . and then moved to -11.5°C ., or frozen and stored for 5 days at -17.8°C . and then moved to -11.5°C ., noticeable separation was delayed from 64 to more than 104 days, from less than 35 to 95 days, and from less than 35 to 84 days, respectively. The appearance of flavor deterioration also was delayed from 47 to 84 days, from 47 to 95 days and from 35 to more than 84 days, respectively.

When the homogenized milk was frozen and stored for 25 days at -27.5°C . and then moved to -17.8°C ., or frozen and stored for 25 days at -27.5°C . and then moved to -11.5°C . or frozen and stored for 25 days at -17.8°C . and then moved to -11.5°C ., sodium citrate plus ascorbic acid prolonged the period before noticeable separation occurred from less than 47 to more than 140 days, from 47

TABLE 3

Influence of sodium citrate and sodium citrate with ascorbic acid on the stability of frozen homogenized milk (Cont'd)

C—Frozen and held at $-27.5^{\circ}\text{C}.$; moved to $-11.5^{\circ}\text{C}.$						
(C ₁) moved after 5 days				(C ₂) moved after 25 days		
Total storage time	Character of sample					
	Control	Sodium citrate	Sodium Citrate with ascorbic acid	Control	Sodium citrate	Sodium Citrate with ascorbic acid
	Sediment					
(days)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)
25	0.10
35	1.00
47	1.90	0.04	0.03	0.50	.	.
55	0.20	2.00	0.10	0.10
64	11.00	0.02	0.10	2.70	0.08	0.10
74	10.80	0.15	0.20	2.00	0.10	0.50
84	10.00	0.50	0.20	9.00	0.40	0.40
95	..	0.40	1.00	.	0.50	0.35
104	.	1.00	0.80	.	0.80	0.45
114	.	.	1.20	.	.	.
123	.	..	2.70	.	.	0.80
140	.	2.40	1.20	.	1.90	2.00
Flavor						
25	Sl. flat
35	Sl. flat
47	Sl. oxidized	Normal	Normal	Sl. oxidized	.	.
55	.	.	Normal	Oxidized	Normal	Normal
64	Sl. oxidized	Normal	Normal	Oxidized	Normal	Normal
74	Sl. oxidized	Normal	Normal	Oxidized	Normal	Normal
84	Sl. oxidized	Sl. flat	Normal	Oxidized	Normal	Normal
95	.	Sl. flat	Sl. flat	.	Sl. flat	Normal
104	..	Flat	Flat	.	Normal	Normal
114	.	.	Sl. flat	.	.	.
123	.	.	Flat	.	.	Normal
140	..	Sl. oxidized	Flat	.	Sl. oxidized	Oxidized
D—Frozen and held at $-17.5^{\circ}\text{C}.$; moved to $-11.5^{\circ}\text{C}.$						
(D ₁) moved after 5 days			(D ₂) moved after 25 days			
Sediment						
25	0.10
35	0.70
47	1.00	..	.	1.0
55	3.00	0.20	.	2.5	..	0.30
64	3.50	0.30	0.30	3.5	0.02	0.40
74	3.00	0.25	0.20	5.0	0.50	0.50
84	..	0.70	0.80	7.0	1.90	1.60
95	.	1.00	.	9.0	1.60	1.80
104	..	1.30	1.60	..	2.50	2.60
114
123	4.5
140	4.8

Flavor						
25	Sl. flat					
35	Sl. oxidized					
47	Sl. oxidized			Tr. oxidized		
55	Oxidized	Normal		Oxidized		Normal
64	Oxidized	Normal	Normal	Oxidized	Normal	Normal
74	Oxidized	Normal	Normal	Oxidized	Normal	Normal
84		Sl. flat	Normal	Sl. oxidized	Sl. flat	Normal
95		Sl. flat		Sl. oxidized	Sl. flat	Tr. oxidized
104		Flat	Flat		Flat	Flat
114						
123					Sl. oxidized	
140					Sl. oxidized	

to 74 days and from less than 47 to 74 days, respectively. The appearance of flavor deterioration also was delayed from less than 47 to more than 104 days, from less than 47 to more than 123 days and from less than 47 to 84 days, respectively. The samples of frozen homogenized milk which contained ascorbic acid in addition to sodium citrate remained normal in flavor slightly longer than the samples containing only sodium citrate.

CONCLUSIONS

Sodium citrate in a concentration of 2 g. per l. was found to be of value as a stabilizer in homogenized milk frozen and stored at -17.8°C . Separation was not noticeable in the thawed milk samples containing sodium citrate until they had been stored for 145 days, and flavor deterioration was not evident until they had been stored for at least 105 days. Noticeable separation had taken place and flavor deterioration had begun in the control samples after a storage period of 65 days.

Homogenized milk containing added ascorbic acid in a concentration of 0.1 g. per l., frozen and stored at -17.8°C ., showed separation when thawed at the same storage age as the control samples, although its addition preserved the flavor slightly longer than did the addition of sodium citrate alone.

Hydrogen peroxide (30 per cent) in a concentration of 1 ml. per l. and urea in a concentration of 3 g. per l. each delayed slightly the occurrence of separation in homogenized milk frozen and stored at -17.8°C . but did not retard the development of abnormal flavors.

Pectin and disodium phosphate, each in a concentration of 1 g. per l., and calcium chloride and carboxyl methyl cellulose, each in a concentration of 0.5 g. per l., did not delay separation or retard the development of abnormal flavors in homogenized milk frozen and stored at -17.8°C .

The addition of sodium citrate with ascorbic acid to homogenized milk, frozen and stored at -11.5°C . and at -17.8°C . doubled the time the milk remained normal in appearance and flavor when thawed in comparison with the control

samples. The addition of sodium citrate with ascorbic acid also prolonged the time that homogenized milk could be frozen and held at one temperature and subsequently stored at a higher temperature and still be normal in appearance and flavor when thawed.

REFERENCES

- (1) BABCOCK, C. J., ROERIG, R. N., STABILE, J. N., DUNLAP, W. A., AND RANDALL, R. Frozen Homogenized Milk. I. Effect of Freezing and Storage Temperatures on the Physical Characteristics of Homogenized Milk. *J. Dairy Sci.*, 29, 10: 699-706. 1946.
- (2) BABCOCK, C. J., ROERIG, R. N., STABILE, J. N., DUNLAP, W. A., AND RANDALL, R. Frozen Homogenized Milk. II. Effect of Freezing and Storage Temperatures on the Chemical and Bacteriological Properties of Homogenized Milk. *J. Dairy Sci.*, 30, 1: 49-54. 1947.
- (3) BABCOCK, C. J., STABILE, J. N., RANDALL, R., AND WINDHAM, E. S. Frozen Homogenized Milk. III. Stability of Milk Solids Distribution in Frozen Homogenized Milk. *J. Dairy Sci.*, 30, 9: 733-736. 1947.
- (4) BABCOCK, C. J., STABILE, J. N., WINDHAM, E. S., EVANS, L. B., AND RANDALL, R. Frozen Homogenized Milk. IV. Keeping Quality of Frozen Homogenized Milk After Thawing. *J. Dairy Sci.*, 31, 9: 805-810. 1948.
- (5) BABCOCK, C. J., STABILE, J. N., WINDHAM, E. S., EVANS, L. B., AND RANDALL, R. Frozen Homogenized Milk. V. Effect of Age Before Freezing on the Keeping Quality of Frozen Homogenized Milk. *J. Dairy Sci.*, 31, 9: 811-815. 1948.
- (6) CIVTL, J. Einfluss des Gefrierens auf einige Bestandteile und Eigenschaften der Milch. *Milchw. Forsch.*, 12: 409-432. 1931.
- (7) DOAN, F. J., AND WARREN, F. G. Observations on the Instability of the Protein Phase of Frozen Concentrated Milk. *J. Dairy Sci.*, 30, 11: 837-848. 1947.
- (8) ROADHOUSE, C. L., AND HENDERSON, J. L. How Quick Freezing Affects Keeping Quality of Milk. *Food Inds.*, 12: 54-55. 1940.
- (9) SEEKLES, L., AND SMEETS, W. TH. G. M. Instability of Milk Due to an Increased Activity of Calcium Ions. *The Netherlands Milk and Dairy J.*, 1, 1: 7-18. 1947.
- (10) TRESSLER, D. K., AND EVERS, C. F. *The Freezing Preservation of Foods*. 2nd Ed. The Avi Publishing Co., Inc., New York, N. Y., p. 630. 1947.
- (11) TROUT, G. M. The Freezing and Thawing of Milk Homogenized at Various Pressures. *J. Dairy Sci.*, 24: 277-287. 1941.
- (12) WEBB, B. H., AND HALL, S. A. Some Physical Effects of Freezing Upon Milk and Cream. *J. Dairy Sci.*, 18: 275-287. 1935.

THE EFFECT OF A COMBINATION OF PENICILLIN AND STREPTOMYCIN UPON THE LIVABILITY AND BACTERIAL CONTENT OF BOVINE SEMEN¹

J. O. ALMQUIST,² P. J. GLANTZ,³ AND H. E. SHAFFER^{2, 4}

Pennsylvania Agricultural Experiment Station, State College

Earlier investigations at this Experiment Station (1, 2) demonstrated that either penicillin or streptomycin will inhibit the growth of bacteria in diluted bull semen. However, concentrations as high as 2,000 units per ml. of diluted semen of either one alone failed to provide complete inhibition. In both studies organisms resistant to the antibiotic employed were encountered in certain of the diluted semen samples.

It generally is recognized that streptomycin is more effective than penicillin against gram-negative microorganisms and that most strains of gram-positive organisms are more sensitive to penicillin than to streptomycin. Since both gram-positive and gram-negative bacteria are found among the predominating types in freshly collected bovine semen (5, 6, 7), it is possible that penicillin and streptomycin in combination would be more effective than either one alone in controlling bacterial growth in diluted semen.

Thompson (8) and Boeing and Laffer (4) found that penicillin plus streptomycin satisfactorily inhibited bacterial growth when added to a selective medium used for the isolation of fungi. Williams and Plastringe (9) reported that the use of 100 units each of penicillin and streptomycin per ml. of medium controlled all bacterial contaminants sufficiently to permit growth of *Trichomonas foetus*. When used alone, a concentration of 1,000 units of streptomycin per ml. was required to free *T. foetus* from *Pseudomonas aeruginosa*. Literature recently reviewed by Beaudette (3) shows that a combination of penicillin and streptomycin was superior to either one alone for controlling bacterial contamination of various exudates and excretions.

The present paper deals with the effect of a combination of penicillin and streptomycin upon spermatozoan livability and the control of bacterial growth in diluted bovine semen.

EXPERIMENTAL

Effect of penicillin-streptomycin upon spermatozoan livability. Based on the livability studies conducted by Almquist *et al.* (1, 2), the following levels

Received for publication September 8, 1948.

¹ Authorized for publication September 1, 1948, as paper no. 1467 in the Journal Series of the Pennsylvania Agricultural Experiment Station. The penicillin and streptomycin were provided by Charles Pfizer and Co., Inc., Brooklyn, New York.

² Dept. of Dairy Husbandry.

³ Animal Pathology Laboratory, Dept. of Animal Husbandry.

⁴ The authors wish to express their appreciation to Dr. W. T. S. Thorp, now with the National Institute of Health, Bethesda, Md., for his suggestions concerning the conduct of the study.

of penicillin (P) and streptomycin (S) with appropriate controls were selected for testing: 100, 250, 500, 750 and 1,000 units of each antibiotic, 250 units of P plus 750 units of S, 750 units of P plus 250 units of S and 500 units of P plus 1,000 units of S per ml. of diluted semen. The preparations used were the sodium salt of penicillin and streptomycin sulphate.

The semen was collected and handled as previously described (1, 2), using a dilution rate of one part of fresh semen to 24 parts of egg yolk-citrate diluter. The dry preparations of penicillin and streptomycin were dissolved in sterile sodium citrate solution and mixed with egg yolk to provide a diluter with 1:1 ratio of yolk to buffer. The diluted semen was stored at 4.5° C., and motility estimations were made every 2 days for a storage period of 20 days. Bacterial plate counts were made on subsamples taken prior to storage and after storage for 8 days.

TABLE 1

The effect of a combination of penicillin and streptomycin upon the livability of bull spermatozoa (Mean of 13 determinations)

Units per ml. of diluted semen		Per cent motile spermatozoa					
Penicillin	Strepto- mycin	Before storage	After storage at 4.5° C. for				
			4 days	8 days	12 days	16 days	20 days
0	0	65	58	43	33	20	8
100	100	65	58	46	34	22	8
250	250	65	59	46	35	22	10
500	500	65	58	44	35	21	9
750	750	65	57	44	35	18	8
1000	1000	65	57	46	33	18	6
250	750	65	57	45	33	20	8
750	250	65	56	46	34	20	8
500	1000	65	59	47	33	20	8

The 13 ejaculates studied had a mean concentration of 1,022,000 spermatozoa per cubic millimeter, a mean initial motility of 65 per cent active spermatozoa, and a mean methylene blue reduction time of 10.6 minutes.

Table 1 gives the mean motility data for the 13 ejaculates. Analysis of variance involving 1170 motility estimations showed no significant difference between treatments. A small but highly significant ejaculate \times treatment interaction was obtained indicating that the various ejaculates responded somewhat differently to the several levels of the antibiotics in their livability during storage.

Procedure used for bacterial studies. Plate counts were determined on undiluted semen, yolk-citrate diluter and diluted semen before storage and after storage for 8 days at 4.5° C. Preparations of materials were timed so that plating procedures could begin as soon as the subsamples were taken. Plating of the fresh material was initiated within 1 hour following treatment with penicillin-streptomycin.

Veal infusion agar containing 4 per cent defibrinated bovine blood was used for determining the approximate numbers of living bacteria. The samples were

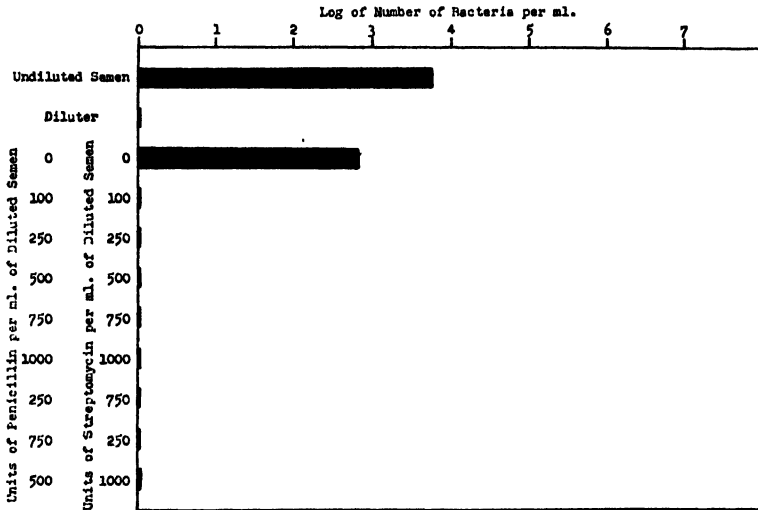


FIG. 1. The effect of a combination of penicillin and streptomycin upon bacterial populations in freshly diluted semen.

plated in dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 and incubated for 48 hours at 37° C. Desoxycholate agar plates incubated for 24 hours at 37° C. were used for determining the number of bacteria belonging to the coliform group. Control plates for sterility tests of the agars and the water used for dilutions also were made.

Effect of penicillin-streptomycin upon bacterial growth in diluted semen.

The effect of penicillin-streptomycin mixtures upon bacterial growth in 5 samples

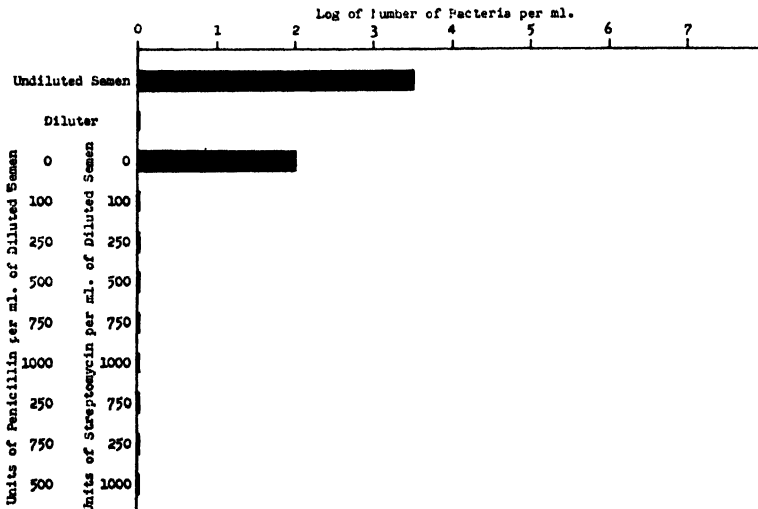


FIG. 2. The effect of a combination of penicillin and streptomycin upon bacterial growth in diluted semen stored for 8 days at 4.5° C.

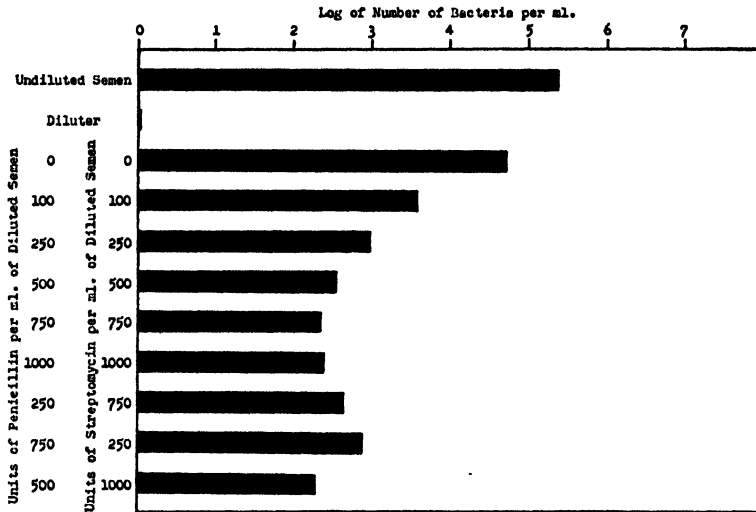


FIG. 3. The effect of a combination of penicillin and streptomycin upon bacterial populations in freshly diluted semen inoculated with bacterial cultures.

of diluted semen is shown in figures 1 and 2. It should be noted that logarithmic rather than arithmetic means have been used to present the mean number of bacteria in the 5 semen samples. Since 1:10 was the lowest serial dilution used and at least 25 colonies were required at this dilution before a count was considered significant, any counts below log number 2.40 indicate only that the material was not sterile.

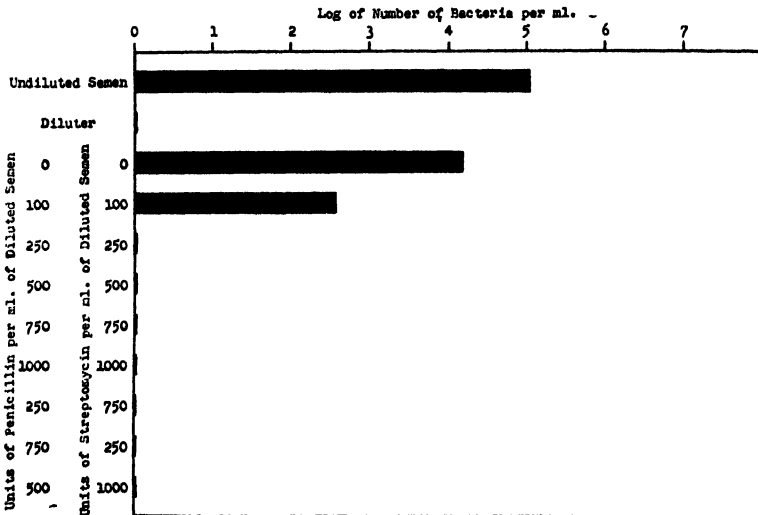


FIG. 4. The effect of a combination of penicillin and streptomycin upon bacterial growth in inoculated, diluted semen stored for 8 days at 4.5° C.

As illustrated in figures 1 and 2, treatment of diluted semen with penicillin and streptomycin together effectively controlled bacterial growth at all levels tested in both fresh and stored semen. No growth was observed on plates representing penicillin-streptomycin treated semen. The desoxycholate agar plates also were negative. However, bacterial contamination of the five fresh, undiluted semen samples was very low, as shown by the average plate count of 5,700 per ml.

Effect of penicillin-streptomycin upon bacterial growth in diluted semen inoculated with bacterial cultures. Because of the relatively low plate counts obtained from the first five undiluted semen samples, the next five samples were inoculated with broth cultures of bacteria. Organisms isolated from previous semen platings on veal infusion blood agar were transferred to veal infusion broth. Since no coliform bacteria were isolated from previous samples of semen, strains of *Escherichia coli* isolated from calf feces were used. Broth cultures of five different types of bacteria, including gram-positive rods, gram-positive cocci and gram-negative rods, were utilized for inoculation of the undiluted semen. After incubation for 24 hours at 37° C., 0.1 ml. of each broth culture was removed to 10 ml. of sterile nutrient broth. This dilution was mixed well and 0.5 ml. transferred to 10 ml. of sterile broth. The latter dilution received directly 0.1 ml. of a 24-hour broth culture of coliform bacteria. After mixing thoroughly, 0.2 ml. of the final dilution was removed to the freshly collected undiluted semen.

The effect of a combination of penicillin and streptomycin upon bacterial growth is illustrated in figures 3 and 4. Figure 3 presents the average plate counts of the freshly diluted, inoculated semen. The undiluted semen had a plate count of 270,000 per ml. (log 5.43), while the diluted semen without the antibiotics averaged 52,000 per ml. (log 4.72). There was a decrease to 4,000 per ml. (log 3.60) when 100 units each of penicillin and streptomycin were added to the diluted semen. The number of bacteria per ml. decreased as the concentrations of penicillin and streptomycin increased. It is possible that better bacterial control of the fresh material was not obtained because the serial dilutions tended to dilute the penicillin-streptomycin concentrations below their most effective inhibitory level.

Figure 4 shows the effect of the combined antibiotics upon bacterial growth in inoculated, diluted semen after storage for 8 days at 4.5° C. There were marked decreases in the bacterial plate counts as compared with the same samples plated before storage. At levels above 100 units each of penicillin and streptomycin, negative plate counts consistently were obtained. At the lowest concentration tested, 100 units of each antibiotic, four of the five samples resulted in negative plates, but one sample gave a significant count of 4,000 per ml. The untreated controls had an average plate count of 16,000 per ml. and the undiluted semen had an average count of 120,000 per ml. after 8 days of storage.

Effect of penicillin-streptomycin upon the growth of coliform organisms. As shown in figure 5, levels of penicillin and streptomycin above 100 units per ml. of each gave almost complete inhibition of organisms belonging to the coliform

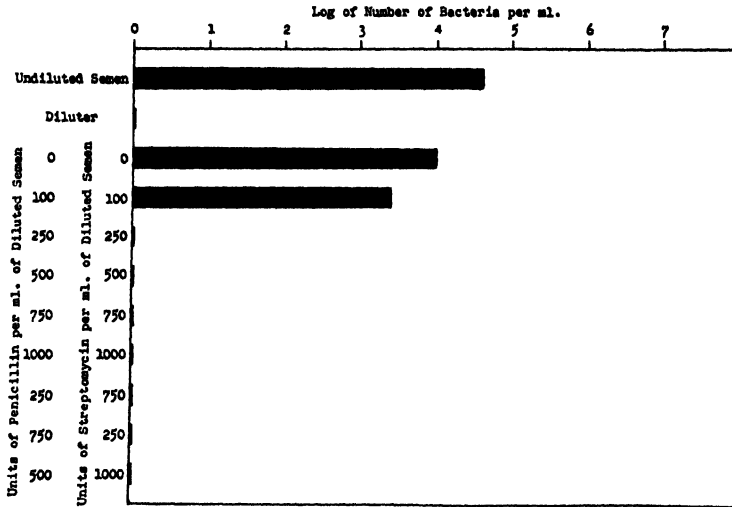


FIG. 5. The effect of a combination of penicillin and streptomycin upon populations of coliform organisms in freshly diluted, inoculated semen.

group. After storage for 8 days, figure 6 shows that all levels of the antibiotics effectively controlled growth of these organisms. When 100 units of each antibiotic per ml. were added, five diluted semen samples showed an average coliform plate count of 2,500 per ml. before storage. However, figure 6 shows that negative counts were obtained at this level when the samples had been stored for 8 days.

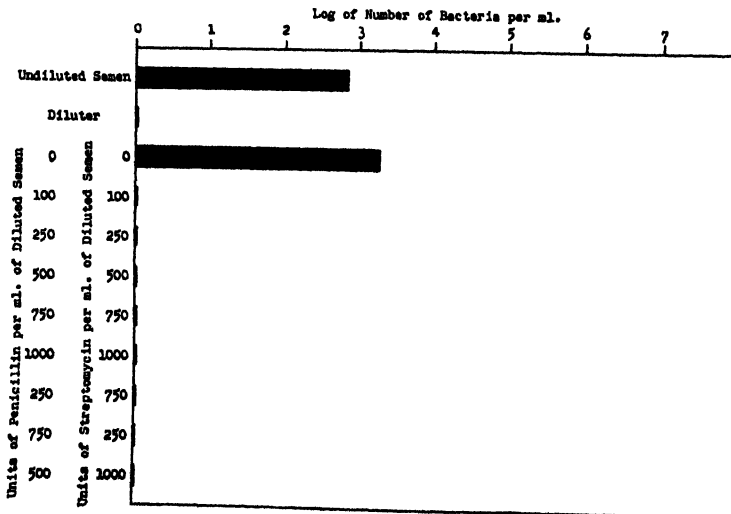


FIG. 6. The effect of a combination of penicillin and streptomycin upon growth of coliform organisms in inoculated, diluted semen after storage for 8 days at 4.5° C.

The coliform plate counts in the undiluted and diluted semen containing no penicillin-streptomycin also decreased during storage. Before storage the inoculated, undiluted semen had a count of 46,000 per ml., while during storage for 8 days there was a marked decrease to 740 per ml. The untreated, inoculated controls decreased from counts of 11,000 per ml. prior to storage to 2,000 per ml. after 8 days of storage.

DISCUSSION

An earlier report by Almquist *et al.* (2) demonstrated that levels of penicillin over 1,000 Oxford units per ml. of diluted semen were injurious to the livability of bull spermatozoa during the time in which practically all semen is used in routine artificial breeding. In similar studies with streptomycin, Almquist *et al.* (1) showed that levels exceeding 1,000 units per ml. of diluted semen significantly reduced spermatozoan livability during a 20-day storage period. In the present study, a combination of penicillin and streptomycin in concentrations as high as 1,000 units of each per ml. of diluted semen did not reduce significantly the ability of the spermatozoa to maintain motility during a storage period of 20 days. Apparently penicillin and streptomycin act independently in their effects upon livability, as the total effect when used together appears to be no greater than when either is used alone.

In comparison with the results of previous studies at this station (1, 2), the use of a combination of penicillin and streptomycin definitely is more effective for controlling bacterial growth in diluted bull semen than either one alone. The results of combined treatment with penicillin and streptomycin showed a marked complementary effect. In spite of inoculation with gram-positive and gram-negative bacteria prior to storage, penicillin-streptomycin treated semen consistently gave negative bacterial counts when plated following storage for 8 days. Field studies now in progress also indicate that negative plate counts may be expected when diluted semen used for artificial breeding is treated with 1,000 units per ml. of both penicillin and streptomycin.

Penicillin and streptomycin assays were not determined in this study. However, Almquist *et al.* (1, 2) found that when used separately these antibiotics were very stable in stored diluted semen. There was no significant loss in streptomycin activity and only a slight decrease in penicillin concentration in diluted semen stored for as long as 16 days. Fertility experiments using penicillin and streptomycin, alone and in combination, are in progress and will be reported as soon as the data are complete.

SUMMARY

1. The use of a combination of penicillin and streptomycin in levels ranging from 100 to 1,000 units of each per ml. of diluted semen did not affect significantly the livability of bull spermatozoa during a 20-day storage period.

2. The use of a combination of penicillin and streptomycin effectively controlled bacterial growth in diluted bull semen.

REFERENCES

- (1) ALMQUIST, J. O., GLANTZ, P. J., AND THORP, W. T. S. The Effect of Streptomycin upon the Livability and Bacterial Content of Bovine Semen. *J. Dairy Sci.*, 31: 501-507. 1948.
- (2) ALMQUIST, J. O., THORP, W. T. S., AND KNOTT, C. B. The Effect of Penicillin upon the Livability, Glycolysis, and Bacterial Content of Bovine Semen. *J. Dairy Sci.*, 31: 11-19. 1948.
- (3) BEAUDETTE, F. R., BIVINS, J. A., AND MILLER, BARBARA RHODES. Use of Antibiotic Agents for Bacterial Sterilization of Respiratory Exudates from Naturally Infected Cases of Newcastle Disease. *Am. J. Vet. Research*, 9: 97-101. 1948.
- (4) BOEING, P. J., AND LAFFER, N. C. Medium for Isolation of Pathogenic Fungi. *Bull. U. S. Army Med. Dept.*, 5: 368. April, 1946.
- (5) GUNSALUS, I. C., SALISBURY, G. W., AND WILLETT, E. L. The Bacteriology of Bull Semen. *J. Dairy Sci.*, 24: 911-919. 1941.
- (6) HATZIOLOS, B. Untersuchungen über Konservierung von Bullensperma zum Zwecke der künstlichen Besamung. *Z. Zücht.*, 38B: 199-254. 1937.
- (7) OGNIANOV, A. La Carica Batterica nello Sperma Bovino Ottenuto Strumentalmente nella Pratica della Fecondazione Artificiale. (The Bacterial Content of Bovine Semen Collected for Artificial Insemination.) Istituto Sperimentale Italiano "L. Spallanzani" per la Fecondazione Artificiale. (Milano), Pp. 48-49. 1942. (*Animal Breeding Abstracts*, 15: 86-87. 1947.)
- (8) THOMPSON, L. Notes on a Selective Medium for Fungi. *Proc. Staff Meetings Mayo Clinic*, 20: 248-249. 1945.
- (9) WILLIAMS, L. F., AND PLASTRIDGE, W. N. Use of Antibiotic Substances for Freeing *Trichomonas foetus* from Bacteria. *J. Bact.*, 51: 127. 1946.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

MARCH, 1949

NUMBER 3

ASH ALKALINITY OF DRY BUTTERMILK

D. W. WHITMAN AND P. H. TRACY

Department of Food Technology, University of Illinois, Urbana

This study was undertaken to determine what standard, if any, could be established to differentiate dry buttermilk manufactured from cream to which a neutralizer had been added from dry buttermilk manufactured from cream to which no neutralizer had been added.

METHODS

The ash alkalinity of the dry buttermilk was determined according to the method of Hillig (1). The source of all samples was cream from milk produced by the University herd. One-gallon samples of 35 per cent cream to be ripened were placed in 5-gallon milk cans, inoculated with 1 per cent butter culture and incubated at 70 to 72° F. in a water bath until the desired acidity was developed. The acidity was raised approximately that percentage which the neutralizer would lower it on a theoretical basis, calculating all acidity as lactic acid. When the desired acidity was developed, the calculated amount of neutralizer was added and the sample was pasteurized at 180° F. for 30 minutes. Some of the samples in which a starter was growing actively increased in acidity while they were being heated for pasteurization. Thus the developed acidity in some samples sometimes was higher than was intended.

Those samples which developed acidity and were not neutralized could not be pasteurized because the high concentration of acid would coagulate the protein. Therefore, the development of acid was checked in these samples by removing them from the water bath and placing them in a hardening room at about -15° F.

The data were analyzed statistically by the student "t" function method (3).

RESULTS

In this study, the ash alkalinity of 21 samples of dry sweet cream buttermilk to which no neutralizer had been added ranged from 6 to 92, with an arithmetic mean of 37 and a geometric mean of 35 (table 1). Fourteen samples gave values in the range from 0 to 49 while 7 were in the range from 50 to 99.

The ash alkalinity of 21 samples of unneutralized dry sour cream buttermilk ranged from 10 to 108 with an arithmetic mean of 49 and a geometric mean of 40. Twelve samples gave values in the range 0 to 49, 7 in the range from 50 to 99 and 2 in the range from 100 to 149 (table 1).

Received for publication August 5, 1948.

TABLE 1

Effect of developed acidity upon the ash alkalinity of dry buttermilk

Sample	A.A.V.* before acidity was developed	A.A.V.* after acidity was developed	% increase in acidity
1	54	50	0.51
2	50	105	0.47
3	25	52	0.51
4	32	35	0.54
5	18	62	0.62
6	30	40	0.65
7	27	48	0.58
8	23	38	0.59
9	36	34	0.63
10	21	22	0.56
11	6	38	0.52
12	27	26	0.57
13	32	10	0.45
14	15	28	0.43
15	20	12	0.46
16	23	10	0.39
17	58	58	0.57
18	68	72	0.60
19	52	98	0.61
20	92	108	0.61
21	78	88	0.50
Arithmetic mean	37	49	
Standard deviation	21	30	
Geometric mean	35	40	

* A.A.V. = Ash alkalinity value.

A statistical analysis of these data indicate 85 per cent certainty that samples containing developed lactic acid will possess higher ash alkalinity values than samples with no developed lactic acid. Therefore differences of this magnitude may be expected solely through errors of random sampling 15 times in 100.

Seven experimental trials were made in which each sample of cream was split into subsamples, ripened, neutralized the desired amount, pasteurized,

TABLE 2

Effect of neutralizing with varying amounts of sodium bicarbonate on ash alkalinity values of dry buttermilk

Sample	Per cent lactic acid neutralized					
	0.00	0.10	0.20	0.30	0.40	0.50
	Ash alkalinity values					
S0	41		190		371	
S2	54	124	142	231	296	356
S3	50	92	152	208	255	355
S4	25	70	120	172	262	390
S5	12	62	132	260	260	385
S6	18	92	162	212	292	348
S7	30	95	165	220	288	396
Arith. mean	33	89	152	217	289	371
Geom. mean	29	87	150	216	287	371

TABLE 3

Effect of neutralization with varying amounts of sodium bicarbonate on the frequency distribution of ash alkalinity values of dry buttermilk

Ash alkalinity values	No. of samples in each neutralization group (Neutralization expressed as per cent lactic acid)						0.50
	0.00 ^a	0.00 ^b	0.10	0.20	0.30	0.40	
0-49	17	12					
50-99	7	7	5				
100-149		2	1	6			
150-199				7	1		
200-249				1	4		
250-299					1	6	
300-349							1
350-399						1	5
Total samples	24	21	6	14	6	7	6
Arith. means ^c	36	49	89	158	217	289	372
Geom. means ^c	30	40	87	154	216	287	371

^a No developed acidity.

^b Developed acidity.

^c Means of ash alkalinities.

cooled, held overnight, churned and dried. Sodium bicarbonate, the only neutralizer added, was used in quantities theoretically needed to neutralize 0.10, 0.20, 0.30, 0.40 and 0.50 per cent lactic acid. The data are presented in table 2. The arithmetic means of the ash alkalinities were 89, 152, 217, 289 and 371, respectively. None of the samples to which neutralizer had been added had ash alkalinity values falling in the range 0-49 (table 3). However, there were 12 such samples that had ash alkalinity values in the range 50-149. All of the ash alkalinity values of samples to which no neutralizer had been added fell within the range 0-149. All samples to which 0.10 per cent neutralizer had been added

TABLE 4

Effect of addition of various neutralizers on ash alkalinity values of dry buttermilk^a

Sample No.	Cream acidity		Ash alkalinity values							
	Before ripening	Before churning	No neutralizer		MgO	MgCO ₃	CaO	CaCO ₃	K ₂ CO ₃	NaHCO ₃
	(%)	(%)	Un-ripened	Ripened						
N0	0.13		21		200	164	173	194	144	120
N1	0.10		40		125	132	119	98	114	128
N2	0.13	0.50	58	58	148	168	170	175	115	148
N3	0.13	0.45	68	72	185	162	195	162	158	182
N4	0.13	0.60	52	98	132	172	175	185	142	178
N5	0.13	0.60	92	108	195	190	190	232	188	200
N6	0.11	0.39	78	88	185	178	182	140	145	158
Arithmetic mean			58	85	167	167	172	169	144	159
Geometric mean			53	83	164	166	170	164	142	157
Standard deviation			24	20	31	18	27	42	25	29

^a The acid of each cream was reduced 0.20 per cent after ripening and before pasteurization by the addition of the neutralizer indicated.

and six of the samples to which 0.20 per cent neutralizer had been added also fell within this range.

Seven lots of cream were used in the study of dry sour cream buttermilk in which the acidity was standardized with various neutralizers. Each lot of cream was divided into two parts. One sub-sample of each was churned and the buttermilk dried. The other part was ripened to give approximately 0.20 per cent additional acidity, after which that amount of neutralizer was added which was theoretically sufficient to neutralize 0.20 per cent lactic acid. The cream was churned and the buttermilk dried. The neutralizers used were magnesium oxide, magnesium carbonate, calcium oxide, calcium carbonate, potassium carbonate and sodium bicarbonate. The results of the ash alkalinity determinations on the dry buttermilk are shown in table 4.

A statistical comparison of ash alkalinity values of samples containing 0.20 per cent lactic acid neutralized with magnesium oxide and samples containing 0.20 per cent lactic acid neutralized with sodium bicarbonate indicates that in less than 55 cases out of 100 will the former have higher ash alkalinities than the latter. A similar comparison of samples neutralized 0.20 per cent with equivalent amounts of calcium oxide and sodium bicarbonate indicates that in 97 cases out of 100 the former will have higher ash alkalinities than the latter.

DISCUSSION

Kunkel and Combs (2) found that the range of ash alkalinity values for 16 samples of dry sweet cream buttermilk to which no neutralizer had been added was from 55 to 112, with an arithmetic mean of 78. In this study, the ash alkalinity values of 21 such samples ranged from 6 to 92 with an arithmetic mean of 37.

A statistical analysis indicated it was only 85 per cent certain that samples containing developed lactic acid will possess a higher ash alkalinity value than samples containing no developed lactic acid.

It also was indicated statistically that it was only 87 per cent certain that samples containing 0.10 per cent neutralized lactic acid would have higher ash alkalinity values than samples containing developed acid but no neutralizer.

Whether this degree of reliability is satisfactory or not depends on how it is used. If used merely as an indicator of quality with an awareness of its limitations, it is satisfactory; if used for regulatory work, a test which fails to identify properly 13 samples out of every 100 hardly could be called satisfactory.

The addition to liquid buttermilk of magnesium oxide, magnesium carbonate, calcium oxide, calcium carbonate, potassium carbonate and sodium bicarbonate sufficient to neutralize 0.20 per cent lactic acid increased the ash alkalinities of the dry buttermilks.

The degree of reliability is such that 99 times in 100 a sample which has had 0.20 per cent lactic acid neutralized by any one of these six compounds will have a higher ash alkalinity value than the same sample containing developed lactic acid but no neutralizer would have had.

In all cases where an ash alkalinity value of 150 or higher was obtained, a neutralizer had been added to the buttermilk before drying.

An ash alkalinity value of 149 or less would include all the samples to which no neutralizer had been added, all samples to which 0.10 per cent neutralizer had been added and approximately one-half of the samples to which 0.20 per cent neutralizer had been added. All samples neutralized more than 0.20 per cent would be excluded from this group.

CONCLUSIONS

From the data obtained on dry buttermilks secured from cream separated from University milk of high quality, it is evident that it is not possible to select a definite ash alkalinity value which will exclude all samples containing added neutralizers and which will include only those samples containing no added neutralizers. This is to be expected because the ash alkalinity values of non-neutralized dry sweet cream buttermilks cover a rather wide range.

Statistical analyses on the samples reported indicate that it would not be possible to distinguish samples of dry buttermilk containing 0.10 per cent neutralized lactic acid from non-neutralized samples of dry buttermilk by means of this test in over 90 cases out of 100.

ACKNOWLEDGMENT

Grateful acknowledgment is made to the American Dry Milk Institute, Inc., of Chicago, Illinois, for the financial aid which made this study possible.

REFERENCES

- (1) HILLIG, F. Report on Neutralizers in Dairy Products. J. Assoc. Offic. Agr. Chemists, 25: 610-612. 1942.
- (2) KUNKEL, R. W. AND COMBS, W. B. The Alkalinity of Milk Ash and Its Relation to the Detection of Neutralizers in Dry Milk. II. Dry Buttermilks. J. Dairy Sci., 28(3): 227-232. 1945.
- (3) TRELOAR, A. E. An Outline of Biometric Analysis. Burgess Publishing Co., Minneapolis. 1936.

THE INFLUENCE OF TOCOPHEROLS AND COD LIVER OIL ON THE STABILITY OF MILK¹

VLADIMIR N. KRUKOVSKY, J. K. LOOSLI, AND FRANK WHITING

Departments of Dairy Industry and Animal Husbandry, Cornell University, Ithaca, N. Y.

In the course of studies relating to the possible cause of the effect of cod liver oil in depressing the fat percentage of milk (8) samples of milk were obtained to test interrelationships between the fat constants and lipolysis of the milk fat. It was observed that the depressing effect of cod liver oil on fat production and the resulting high iodine numbers might not necessarily be permanent. In other words, the cow may adjust to this condition of feeding, and the fat production and the iodine numbers² may return to normal within a cod liver oil feeding period.

Since these observations also bear a relationship to the susceptibility of milk to the development of the oxidized flavors, it was thought of interest to present them together with more recent data on the influence of tocopherol and cod liver oil on the milk and fat production (12) and the stability of milk.

EXPERIMENTAL

The original data on the effects of the addition of cod liver oil to the feed of cows and of subsequent drenching on the iodine numbers and the susceptibility of milk to the development of oxidized flavors are presented in figure 1. Two cows were used in this experiment. From November 6 to 30 (period A) both cows were fed 0.5 ml. of a commercial grade cod liver oil per kilogram body weight by mixing it in the feed (dry beet pulp) (8). At the end of this period, the same amount of cod liver oil was administered to cow no. 1 by drench; this was continued until December 16 (period B), while cow no. 2 received no cod liver oil.

The data present in figure 1 show that from November 6 to 30 (period A) both the iodine numbers and, to some extent, the fat production returned to normal after an ecliptic rise or drop in their values for both cows. The following drench feeding of cod liver oil, from December 1 to 16 (period B), apparently destroyed the ability of the cow no. 1 to readjust physiologically to this feeding condition. The resulting rise in iodine values of the fat was, however, much more gradual than at the beginning of the experiment. Although these data are not sufficient to warrant a definite conclusion, they show a possibility that the conditions of feeding and the length of the feeding period both are responsible for the physiological behavior of the cow. It should be noted, how-

Received for publication August 26, 1948.

¹ This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned no. 218 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

² Hannus method (1).

ever, that the samples of milk were collected and analyzed daily, and that the foregoing effect would be masked if composite samples of milk were collected and analyzed.

It further was noticed that the development of the oxidized flavors in fresh pasteurized milk³ obtained from the cows on the cod liver oil feeding trial varied directly with the iodine numbers of the fat. The data in figure 1 show that the drop in the iodine numbers of the fat resulted in the retardation of the development of oxidized flavors, whereas the rise in the iodine numbers during the drench period stimulated the development of oxidized flavors.

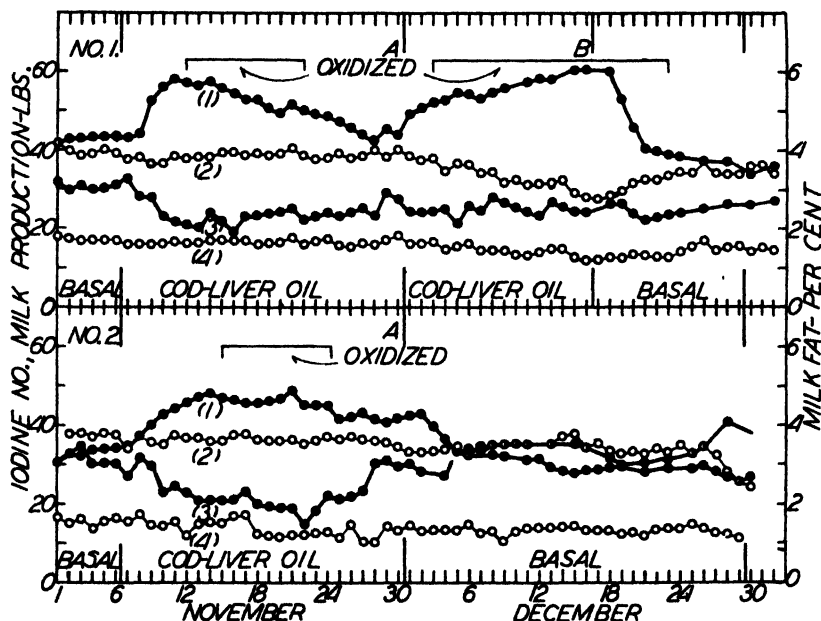


FIG. 1. The effects of the addition of cod liver oil to the feed of the cow (No. 1-A and No. 2) and of the subsequent drenching (No. 1-B) on iodine number, fat content and the susceptibility of milk to development of the oxidized flavors. The symbols indicate: (1), iodine numbers; (2), total milk production; (3), milk fat—per cent; (4), morning milk production.

Since our observations have indicated that there might be a relationship between the vitamin E content of the fat, as affected by seasonal variations of the feed (primarily pasture and hay feeding) (6, 7), and the susceptibility of fresh milk to the development of oxidized flavors, it was thought of importance to learn if the depressing influence of cod liver oil upon the tocopherol content of fat was partially responsible for the inability of milk to resist oxidation.

Consequently, the milk samples obtained from 16 dairy cows of Holstein, Brown Swiss and Guernsey breeds were used for the studies of the influence of tocopherols and cod liver oil on milk fat production and the natural ability of

³ Milk was pasteurized at 61.6° C. for 30 minutes and then held at 0 to 5° C. for 7 days.

milk to resist the oxidative deterioration. One gram of mixed natural tocopherol and 28.35 g. of veterinary grade cod liver oil were added, either alone or together, to a control ration. The detailed procedure for this study is described in the preceding paper (12).

The feeding of cod liver oil alone caused a decrease in average tocopherol content of the fat (from 2900 $\mu\text{g.}$ to 2529 $\mu\text{g./100 g. fat}$), and when the tocopherol and cod liver oil were fed together the average tocopherol content of the milk fat (3590 $\mu\text{g./100 g. fat}$) remained essentially the same as when the tocopherol was fed alone (3569 $\mu\text{g./100 g. fat}$).⁴

The data presented in figure 2 show the per cent distribution of tocopherols in the samples of stable and unstable milks as affected by tocopherol and cod

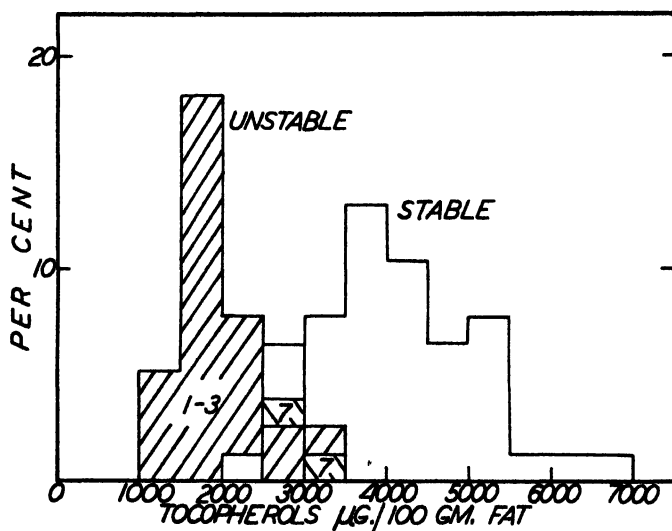


FIG. 2. The distribution of tocopherols in 77 samples of stable and unstable milk as affected by tocopherol and cod liver oil supplements. (The numbers (1-3 and 7) indicate the days required for oxidized flavor development in unstable milk.)

liver oil supplements. The stability of milk was determined on the basis of its ability to resist the reaction which produces oxidized flavors during a storage period of 7 days at 0 to 5° C.

A highly significant correlation (+0.51) was found between the tocopherol content of the milk fat and the ability of milk to resist development of oxidized flavors. The data also reveal that by varying the tocopherol content of the milk fat through feeding the stability of the fresh pasteurized milk was improved when its tocopherol content was increased to 3000 $\mu\text{g.}$ per 100 g. of fat and above. All such samples were stable during the 7-day experiment. On the other hand, the milk containing less than 2000 $\mu\text{g.}$ tocopherols per 100 g. of fat showed poor keeping qualities with respect to the development of oxidized

⁴ Vitamin A and E were determined using Koehen and Sherman (2) and Quaife (9) methods, respectively.

flavors. This milk could be improved, however, by destroying its total vitamin C.

Furthermore, a relationship also was indicated between the tocopherol content of milk fat, as influenced by various hays, and the stability of milk (7), and between the tocopherol and carotenoid content of milk fat during pasture and winter feeding (6).

In a preliminary study concerning the palatability of hay, the deposition of fat-soluble vitamins in the milk fat and the stability of milk, 15 Holstein cows were fed six types of hay in an incomplete block design experiment. It was found that ladino clover hay and late-cut timothy hay in the rations were responsible for a decrease in the vitamin content of the fat (average values per 100 g. of fat: carotene, 347 $\mu\text{g.}$; vitamin A, 438 $\mu\text{g.}$; tocopherols, 1,873 $\mu\text{g.}$). On the other hand, the feeding of birds-foot trefoil hay, which appealed to the cows more than any other hay, resulted in an appreciable increase in the content of vitamins A and E in the fat (average values per 100 g. of fat: carotene, 723 $\mu\text{g.}$; vitamin A, 703 $\mu\text{g.}$; tocopherols, 2,952 $\mu\text{g.}$), possibly bringing it up to the level observed during pasture feeding.

Milk of poor keeping quality resulted during the ladino clover feeding and could be correlated with the low content of tocopherol, suggesting a possibility that the development of the oxidized flavors in the milk is caused by the type of hay or other roughages fed. This study, however, will be repeated to obtain more information concerning the influence of hay upon the vitamin content and the keeping quality of milk.

Thus, it would appear that the increase of the reducing power of fat, as determined by the tocopherol method, might result not only in the stabilization of market milk, but also in better nutritional properties with respect to vitamins E and A.

DISCUSSION

There apparently are two systems in the milk which are antagonistic to each other: one involves the oxidation of ascorbic acid and of the unstable lipid fraction of the milk (3), and the other is represented by the antioxidant activity of the fat itself. The authors believe that the antioxidant activity of the fat, as determined by the vitamin E method (9) (reducing power), is an important factor with respect to the stabilization of both the fat and the unstable lipid fraction of the milk. The latter is a part of the fat-globule-stabilizing membrane. It is known that buttermilk containing the materials adsorbed on the surface of the fat globules undergoes oxidative deterioration in the presence of ascorbic acid at a much faster rate and to a greater extent than does fresh milk (3, 4). It would be logical to assume, therefore, that the antioxidant activity centered in the fat phase of the milk might exert a protective influence not only to the fat itself but also to the materials which are adsorbed on the surface of the fat globules.

A private communication from Betty M. Watts (11) supports our observations concerning the role played by ascorbic acid and tocopherols in stimulation

or inhibition of oxidative rancidity in edible fats. Their study was concerned with the acceleration by ascorbic acid of oxidative rancidity in rendered pork fat. They found that the acceleration changed to inhibition as the level of tocopherols was raised.

In this connection it should be noted that the rate of ascorbic acid oxidation is a primary factor responsible for the development of oxidized flavors in fresh milk (4, 5). This oxidation of ascorbic acid often is accompanied by a gradual increase in Eh factor up to the point when all of the ascorbic acid is oxidized to dehydroascorbic acid, and then the Eh declines, finally attaining approximately its original value. This process could be repeated by the readdition of ascorbic acid. For example, the photo-oxidation of ascorbic acid to dehydroascorbic acid was found to be accompanied by a rise in Eh factor (10).

The removal of the catalyst (light) at any point along the line, but prior to completion of ascorbic acid oxidation, results invariably in a temporary stability in the Eh factor. The following changes in Eh would depend primarily upon the rate of ascorbic acid oxidation. Finally, when all of the ascorbic acid is oxidized, the Eh value might stabilize itself at the level approaching that of the original system.

It has been shown that the partial photo-chemical oxidation of ascorbic acid to dehydroascorbic acid stimulates the reaction which produces oxidized flavors in milk, whereas its complete oxidation either retards or prevents the reaction (4, 5). It would appear, therefore, that the Eh should be considered only as the by-product of the reaction involving ascorbic acid oxidation. It could not be considered, under any circumstances, as a criterion of the ability of fresh milk to resist the reaction which produces oxidized flavors.

SUMMARY

A significant correlation was found between the tocopherol content of milk fat and the ability of milk to resist the reaction, involving ascorbic acid oxidation, which produces oxidized flavors. This might explain the differences between the stabilities of winter and summer milks.

REFERENCES

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 4th ed. Washington, D. C. 1935.
- (2) KOEHN, C. J., AND SHERMAN, W. C. The Determination of Vitamin A and Carotene with the Photoelectric Colorimeter. *J. Biol. Chem.*, 132: 527-538. 1948.
- (3) KRUKOVSKY, V. N. The Factors Affecting the Stability of Milk Fat and Fat Soluble Vitamins. Paper presented at the Annual Meeting of the American Dry Milk Institute, Chicago, Ill. A.D.M.I., Vol. 2, no. 2. May, 1948.
- (4) KRUKOVSKY, V. N., AND GUTHRIE, E. S. Ascorbic Acid Oxidation, A Key Factor in the Inhibition or Promotion of the Tallowy Flavor in Milk. *J. Dairy Sci.*, 28: 565-579. 1945.
- (5) KRUKOVSKY, V. N., AND GUTHRIE, E. S. Vitamin C, Hydrogen Peroxide, Copper and the Tallowy Flavor in Milk. *J. Dairy Sci.*, 29: 293-306. 1946.
- (6) KRUKOVSKY, V. N., AND WHITING, F. Covitamin Studies of the Milk Fats from 4 Breeds of Dairy Cattle. (Abs.) *J. Dairy Sci.*, 31: 666. 1948.

- (7) LOOSLI, J. K., KEUKOVSKY, V. N., AND LORGREEN, G. P. The Influence of Various Hays on the Production, Vitamin Content and Flavor of Milk. (Abs.) J. Dairy Sci., 31: 690. 1948.
- (8) MCCAY, C. M. Unpublished data, Cornell Univ., Ithaca, N. Y. 1939.
- (9) QUAIFFE, M. L. Tocopherols (Vitamin E) in Milk: Their Chemical Determination and Occurrence in Human Milk. J. Biol. Chem., 169: 513-514. 1947.
- (10) SNYDER, JEANETTE BEULAH. Factors Affecting the Lag Phase in Bacterial Growth. Unpublished thesis, Cornell Univ., Ithaca, N. Y. 1948.
- (11) WATTS, BETTY M. Private communication. Dept. of Foods and Nutrition. Syracuse Univ. June, 1948.
- (12) WHITING, F., LOOSLI, J. K., KEUKOVSKY, V. N., AND TURK, K. L. The Influence of Tocopherols and Cod-Liver Oil on Milk and Fat Production. J. Dairy Sci., 32: 133-138. 1949.

THE EFFECT OF HANDLING AND PROCESSING OF MILK ON ITS OXYGEN CONTENT

E. O. HERREID AND J. FRANCIS

Department of Food Technology, University of Illinois, Urbana

The effect of dissolved oxygen on the flavor and keeping quality of milk has been shown by a number of investigators. The purpose of this study was to determine the effect of various handling and processing operations on the oxygen content of market milk.

REVIEW OF LITERATURE

The oxygen content of anaerobically-drawn milk has been reported in volumes per cent as 4.29 by Hoppe (5), as 1.93 and 4.79 by Stechnow (12), as 1.19 and 1.06 by Pfluger (8), as 2.88, 3.09, 2.69, 2.14, 1.60 and 2.12 by Marshall (6), and as 1.17 by Frayer (2). On the other hand, Guthrie (3), Guthrie *et al.* (4) and Sharp *et al.* (9) reported that milk in the udder of the cow is practically devoid of oxygen. Hoppe's (5) oxygen values were obtained from goat's milk. It is presumed that the values reported by all the other investigators were obtained from cow's milk.

Guthrie *et al.* (4) reported that machine-milked milk contained 1 to 2 mg. of oxygen per liter less than that milked by hand. They found that milk dissolved 3.84 to 9.74 mg. of oxygen per liter during milking. Frayer (2) and Noll and Supplee (7) reported that the oxygen content of raw milk varied from 0.40 to 0.50 volumes per cent. Sharp *et al.* (9) found that 40 cans of raw milk received in a plant in New York City contained 4 to 7.1 mg. of oxygen per liter in samples which represented over half a million pounds of raw milk, and in another trial (10) they reported 9.3 to 11.7 mg. of oxygen.

EXPERIMENTAL PROCEDURE

Four plants, designated as *A*, *B*, *C* and *D*, were selected for this study. Plant *A* is the University of Illinois Creamery where milk is obtained from the University farm, while the other plants are located in the Champaign-Urbana area.

Plant A. In this plant ten trials of 1 day each were completed in order to determine how closely the results from each trial would agree and thus provide a basis for establishing the minimum number of trials that would be necessary in similar experiments in other plants.

Samples were collected from the same ten animals for 1 day at the farm. A different group of ten cows was used in each of the ten trials which were conducted during the period from January 15 to March 1. Samples were taken in the morning and in the evening from each of the cans as they were filled with milk from the surface cooler at 8 to 10° C., and they were held at the farm and

Received for publication Sept. 9, 1948.

delivered to the processing plant at or near these temperatures. When the milk arrived at the processing plant, samples were taken from the following points: weighing tank, preheater at 27 to 30° C., clarifier at the discharge side, pasteurizer at 38 to 39° C., milk pasteurized at 66° C. for 30 minutes and before it was cooled, milk immediately after it had passed through homogenizer at 55° F. and at 2,000 lb. pressure, milk at 50 to 53° C. from top of surface cooler, milk at 3 to 4° C. from bottom of surface cooler and before it reached the trough, bowl in the bottle filler at 5° C., bottles immediately after being filled and bottles after storage at 4 to 8° C. for 24 hours.

Samples were collected at plant A for Eh determinations which were made with a Model G Beckman potentiometer. Plate counts were made on the raw and pasteurized samples according to Standard Methods (1).

Plant B. Two trials were made in this plant during the fourth week of March. Two different trucks delivered milk to this plant from producers who averaged two to four 10-gallon cans daily which, in the majority of cases, were filled only partially. The average temperature of the milk at the time of delivery was 7° C.

Samples were taken from the following points: weighing tank from ten producers, filter by draining milk at 7 to 10° C. through valve, storage tank where filtered milk was held at 8 to 10° C., plate heater just before milk passed through, plate heater, after milk at 68 to 72° C. had passed through, clarifier, as milk at 71° C. was discharged, milk immediately after it passed through homogenizer at 69 to 70° C., at 1,500 lb. pressure, from three 200-gallon holding vats with milk at 65 to 67° C., plate cooler, where milk was cooled at 9 to 10° C., bottle filled with milk at 9 to 10° C., bottles immediately after being filled and bottles after storage at 4 to 8° C. for 24 hours.

Plant C. In this plant, two trials were conducted during the first week of April. This plant received one truck load of milk from patrons, each of whom delivered two to three cans of milk which were not completely full in all cases. The temperatures of the milk varied from 9 to 27° C.

Samples were taken from the following points: weighing tank, storage tank with milk at 17 to 20° C., preheater with milk at 43° C., clarifier as milk at 41° C. was discharged, pasteurizing vat with milk at 49 to 52° C., milk pasteurized at 65° C. for 30 minutes, milk immediately after it passed through homogenizer at 63 to 64° C. at 2,000 lb. pressure, plate cooler after milk had been cooled at 8° C. bottle filler of vacuum type with milk at 8° C., milk from bottles immediately after being filled and milk from bottles after storage at 4 to 8° C. for 24 hours.

Plant D. Two trials were conducted in this plant during the second week of April. The milk, at 10 to 15° C., was delivered to the plant about 11:30 a.m. and consisted of two to five cans from each producer.

The oxygen determinations of the milk were made at 21 to 25° C. by the method of Sharp *et al.* (11), the only variation being that the tubes in which the samples were collected and the oxygen determinations made were painted black.

Samples of milk were taken at easily accessible points in the processing operations with a 50 ml. pipette. Samples were taken from the closed milk lines through sanitary pipe and fittings to which stopcocks were attached. The milk flowed into the sampling tubes through glass tubes which were attached to the stopcocks with short pieces of rubber tubing. Immediately after the samples were taken at the different processing points, the tubes were stoppered tightly and the contents cooled to 3 to 5° C. in an iced bath. The samples were analyzed for oxygen about 2 to 4 hours after they were taken, except those from the farm of plant A, which were analyzed within 6 hours.

EXPERIMENTAL RESULTS

Plant A. The results of the ten trials showing the dissolved oxygen contents of the milk at each of the processing points are averaged in table 1. Assuming

TABLE 1

The effect of handling and of processing on the oxygen content of milk in plant A

Source of milk	Oxygen content	
	mg./l.	standard error
Pails, P.M. ^a	4.50	± 0.11
Pails, A.M. ^a	4.25	± 0.10
Cans, P.M. ^a	6.76	± 0.20
Cans, A.M. ^a	6.80	± 0.21
Weigh tank ^b	6.83	± 0.22
Preheater ^b	6.86	± 0.22
Clarifier	7.15	± 0.26
Before pasteurization ^b	6.20	± 0.18
After pasteurization ^b	5.06	± 0.11
After homogenization ^b	5.72	± 0.23
Top of cooler ^b	5.70	± 0.24
Bottom of cooler ^b	6.79	± 0.22
Filler ^b	6.73	± 0.15
Bottled ^c	6.59	± 0.20
Bottled, after 24 hours ^c	6.76	± 0.19

^a Average of 10 samples in each trial.

^b Average of 3 samples in each trial.

^c Average of 5 samples in each trial.

there is little, if any, free oxygen in milk in the udder (3, 4, 9), the greatest solution of oxygen occurred at the time of milking. The oxygen content of the cooled milk in the cans increased over that in the pails but remained about the same for both the evening and morning milk. The oxygen contents of the milk in the cans, in the weigh tank and in the preheater do not show any significant differences as indicated by the means and standard error of the means; in fact, the oxygen content increased only slightly at the clarifier. As the milk was heated in the vat before pasteurization, the oxygen content decreased, and it decreased further during pasteurization. The oxygen content increased to 5.72 mg. immediately after homogenization and remained near this level at the top of the cooler. The oxygen contents of the milk samples from the bottom of the cooler, from the filler, from milk bottled immediately, and from that bottled for 24 hours, do not show any significant differences.

Plate counts were made of the raw and pasteurized milk in each of the ten trials and they varied from 4,200 to 48,000 and 300 to 1,300 per ml., respectively, and are insufficient to affect the oxygen content of raw and pasteurized milk measurably.

Oxidation-reduction potential (Eh) determinations were made of the samples of milk taken at the different processing points in all ten trials. The Eh values increased from 301.3 mv. at the time of milking to 311.3 mv. in the freshly-bottled milk, to 315.3 mv. for milk held in the bottles for 24 hours.

Since the standard errors of the average oxygen determinations made at each of the processing points in the ten trials conducted in plant A were fairly consistent and of about the same magnitude (table 1), it was decided that two experimental trials of 1 day each in plants B, C and D would be sufficient.

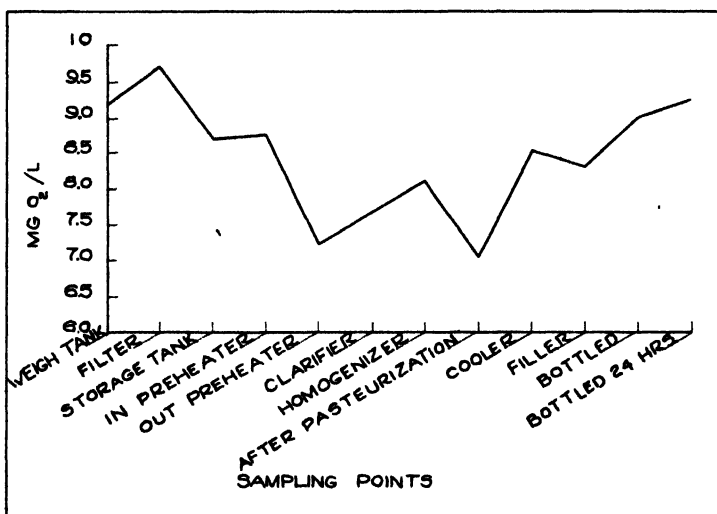


FIG. 1. Average oxygen content of milk at various steps during processing operations in plant B.

Plant B. The raw milk as it arrived at the plant had a relatively high oxygen content which probably was due to agitation of the partially-filled cans of cold milk (figure 1). The oxygen content of the milk increased as it passed through the filter from the weigh can, but at the storage tank it decreased 1 mg. From the storage tank to the preheater, the oxygen content remained about the same, followed by a decrease of 1.5 mg. after preheating, but increased again after clarification. At the homogenizer the oxygen content increased, followed by a substantial decrease in the holding vat after pasteurization. It increased about 1.7 mg. as it left the cooler, dropped slightly at the filler but increased slightly after bottling and after 24 hours storage.

The plate counts of the raw milk in the two trials were 2,200,000 and 2,400,000 per ml. and of the pasteurized milk 27,000 and 41,000 per ml., respectively.

Plant C. The milk delivered to this plant had a lower oxygen content at

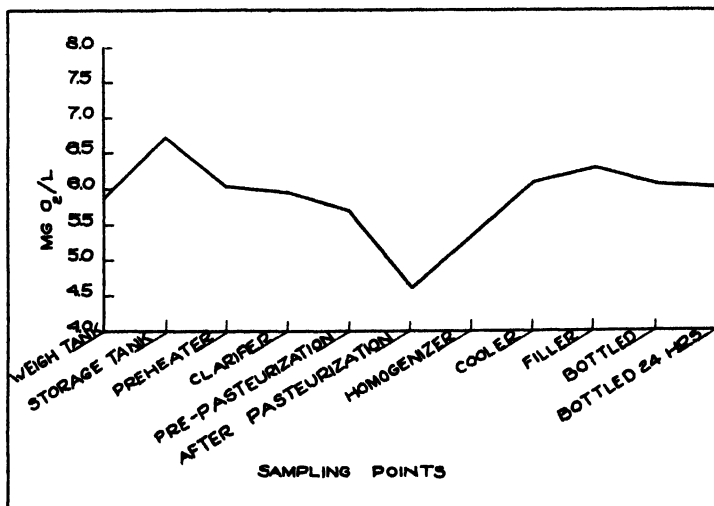


Fig. 2. Average oxygen content of milk at various steps during processing operations in plant C.

the weigh tank than that found at any of the other plants (figure 2). This may be due to the greater counts of bacteria and to the higher temperatures of the milk at the time of delivery.

At the weigh tank the milk had an average oxygen content of 5.84 mg./l. which increased to 6.72 mg. in the storage tank but decreased at the preheater and slightly decreased at the clarifier; the lowest oxygen level occurred during

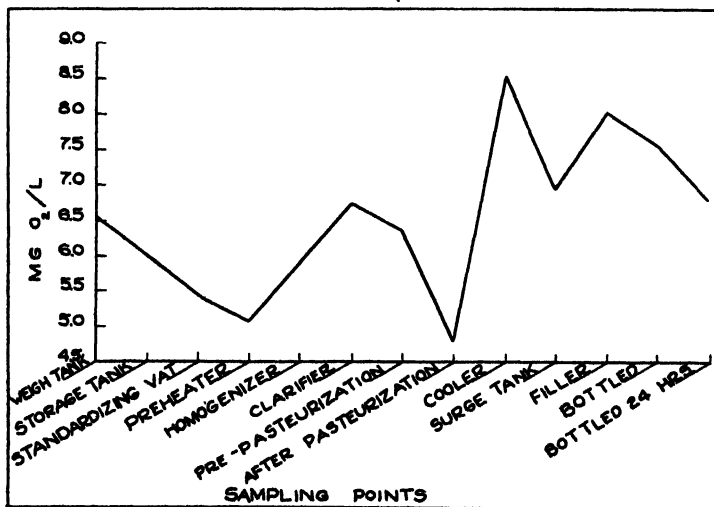


Fig. 3. Average oxygen content of milk at various steps during processing operations in plant D.

pasteurization. The oxygen content of the pasteurized milk increased 0.7 mg. at the homogenizer and increased further after cooling, but remained about the same at the bottle filler and in the bottles.

The plate counts of the raw milk in these trials were 6,500,000 and 6,200,000 per ml., and the pasteurized milk, 130,000 and 67,000 per ml., respectively.

Plant D. The milk at the weigh tank had an average oxygen content of 6.54 mg./l. (figure 3). After being cooled and held in the storage tank, there was a decrease of about 0.5 mg., and a further decrease in the standardizing vat and at the preheater. The oxygen content of the milk increased at the homogenizer, increased further at the clarifier and reached its lowest level during pasturization. As the milk flowed from the cooler, the oxygen content increased nearly 4 mg. but decreased about 2 mg./l. in the surge tank. Milk from the filler bowl showed an increase in oxygen which was followed by decreases immediately after bottling and after storage for 24 hours.

The plate counts of the raw milk in both trials were 500,000 and 470,000 per ml., and the pasteurized milk 9,700 and 9,200 per ml., respectively.

DISCUSSION

Assuming only small amounts of oxygen in milk in the udder, the greatest oxygen absorption occurred at the time of milking. The oxygen content was higher when milk was subjected to agitation in partially-filled cans enroute to the plant.

The same general trend in oxygen content of the milk at similar processing points was observed in all four plants. There was a close relationship in all plants between the oxygen content of the raw milk at the time of delivery and its oxygen content at the various points of processing; however, the percentage decrease or increase at any of these points was approximately the same when calculated on the basis of the oxygen content of the raw milk in the weigh tank.

As expected, the lowest oxygen level occurred in milk during pasteurization, being 21.2 to 27.3 per cent less than the raw milk in the weigh tank, and it increased again when the milk was cooled. In plant *D*, where a high-temperature short-time pasteurizer was used, a reduction in oxygen occurred during pasteurization in this closed system, with a subsequent increase in cooling. The effect of heat on the solution of oxygen in milk conforms to the solubility of gases in liquids as affected by temperature, a fact pointed out by Noll and Supplee (7).

The lower oxygen level in the raw milk in plant *C* probably was due to the higher bacterial count and to the higher temperatures at which most of the raw milk was delivered. Furthermore, it is interesting to note that the oxygen content of the milk at comparable processing points was lower in plant *C* than in any of the other plants.

In all plants, the amount of oxygen in the bottled milk was approximately the same as that in the weigh tank.

CONCLUSIONS

1. The greatest amount of oxygen absorption occurred during milking.
2. The amount of dissolved oxygen in raw milk presumably was influenced by its temperature and bacterial content and by the amount of milk in the cans at the time of delivery.
3. The oxygen content of the milk at various processing points was related to its oxygen content in the weigh tank.
4. The oxygen content in the bottled milk attained an equilibrium comparable to that of the raw milk in the weigh tank.
5. The oxygen content of the milk showed the same general trend at comparable processing points in all four plants.
6. The solubility of oxygen in milk conforms to the solubility of gases in solutions as affected by temperature.

REFERENCES

- (1) AM. PUB. HEALTH ASSOC. Standard Methods for the Examination of Dairy Products. 8th Ed. 1941.
- (2) FRAYER, J. M. The Dissolved Gases in Milk and Dye Reduction. Vt. Agr. Expt. Sta. Bull. 461. 1940.
- (3) GUTHRIE, E. S. The Results of Deaeration on the Oxygen, Vitamin C, and the Oxidized Flavors of Milk. J. Dairy Sci., 29: 359-369. 1946.
- (4) GUTHRIE, E. S., SHARP, P. F., AND HAND, D. B. Oxygen Constant Variants. Am. Milk Rev., 2(6): 131-132. 1940.
- (5) HOPPE, J. Arch. Path. Anat. Physiol. (Virchow's) 17: 417. 1859. (Original not seen. Cited by Marshall (6).)
- (6) MARSHALL, C. E. The Aeration of Milk. Mich. Agr. Expt. Sta. Spec. Bull. 16. 1902.
- (7) NOLL, C. I., AND SUPPLEE, G. C. Factors Affecting the Gas Content of Milk. J. Dairy Sci. 24: 993-1013. 1941.
- (8) PFLUGER, H. Pfluger's Arch. ges. Physiol., 2: 166-173. 1869. (Original not seen. Cited by Marshall (6).)
- (9) SHARP, P. F., HAND, D. B., AND GUTHRIE, E. S. Supplementary Notes on the Deaeration of Milk. J. Milk Technol., 3: 228-229. 1940.
- (10) SHARP, P. F., GUTHRIE, E. S., AND HAND, D. B. Deaeration as a Means of Retarding Oxidized Flavors and Preserving the Vitamin C of Milk. J. Milk Technol., 4: 138-143. 1941.
- (11) SHARP, P. F., HAND, D. B., AND GUTHRIE, E. S. Quantitative Determination of Dissolved Oxygen, Ascorbic Acid Oxidase Method. Ind. Eng. Chem., Anal. Ed., 13 (9): 595-597. 1941.
- (12) STECHNOW, L. Zeit. f. rat. Med. von Henle u. Pfeuffer. 10: 285. 1861. (Original not seen. Cited by Marshall (6).)

PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW.

III. SEVERAL FACTORS AFFECTING VITAMIN A AND CAROTENOID CONTENT^{1, 2}

D. B. PARRISH, G. H. WISE,³ F. W. ATKESON, AND J. S. HUGHES

Kansas Agricultural Experiment Station, Manhattan

Vitamin A and carotenoids in colostrum from dairy cows have been the subject of numerous investigations. It has been observed that levels of these constituents generally are high in the initial colostrum and colostic fat but decrease rapidly as the mammary secretions change to normal milk (4, 6, 8, 11, 14, 20, 21, 22, 25, 26, 27, 28, 29). Similar changes were noted in colostrum from sheep (1, 23, 31) and from women (5, 15, 16). During the early stages of the transition, decreases in concentration of fat-soluble pigments of bovine colostrum have been reported to follow a logarithmic trend (22), but an expression of the rate of change of vitamin A apparently has not been published.

In one study (27), supplementation of the ration of preparturient cows with vitamin A and carotene (carrots) did not appear to increase levels of these constituents in colostrum; however, others found that pasture increased the carotene (11) and the total vitamin A potency (14, 21). Recent investigations have shown that prepartal vitamin A supplementation augmented the vitamin A content of colostrum from cows (7, 25) and from does and sows (30).

Much of the previous work has emphasized the variability in concentrations of vitamin A and carotenoids of colostrum, even from animals maintained under similar conditions. Vitamin A levels of the first postpartum mammary secretions from first-lactation cows were found to be approximately double those from cows in later lactations, but differences have not been reported for carotenoids (4, 10, 11). The effect of breed upon vitamin A and carotenoids of colostrum either has been investigated with too few cows to warrant general conclusions (9, 24) or results have been complicated by inclusion of both first- and later-lactation animals in the same experimental groups (28, 29).

Since it seemed desirable to obtain additional information on effects of various factors on vitamin A and carotenoid levels in colostrum and early milk, the present study was undertaken. Factors investigated were individuality of cows, breed, lactation number, type of prepartal ration and stage in the transition period.

EXPERIMENTAL

Feeding and management of experimental animals. In the major trials, comparisons were made of vitamin A and of carotenoid concentrations in co-

Received for publication September 10, 1948.

¹ Contribution no. 372, Department of Chemistry, and no. 178, Department of Dairy Husbandry.

² Portion of a thesis presented by D. B. Parrish in partial fulfillment of the requirements for the degree Doctor of Philosophy in chemistry at Kansas State College.

³ Present address, Department of Animal Husbandry, Iowa State College, Ames.

lostrum from groups of cows receiving either typical unsupplemented barn feeds or similar rations supplemented with seasonal pasture or vitamin A concentrates. The 86 experimental subjects, which included both first- and later-lactation cows of Holstein, Ayrshire, Jersey, and Guernsey breeds, were divided into three groups. The reference group, designated as the "barn-fed," received a typical barn ration consisting of a concentrate mixture, Atlas sorgo silage and hay, fed according to conventional methods. The "pasture-supplemented" and the "vitamin A-supplemented" groups received, respectively, pasture grasses and vitamin A concentrates⁴ in addition to the barn rations.

The hay fed consisted principally of alfalfa, but some prairie hay also was used. Pasture grazing was supplied by rye, a combination of alfalfa and brome grass, native grass or Sudan grass, depending on availability at different periods.

The trials were designed to restrict all the cows to experimental rations during the major portion of the 6- to 8-week prepartal conditioning period. Duration of the experimental feeding, however, varied considerably, depending upon seasonal conditions and upon deviations from expected date of parturition. In only six cases was the length of the prepartal supplemental-feeding period less than 2 weeks. Cows in barn-fed groups were off pasture 2 to 6 months prepartal. Supplementation with either pasture or vitamin A was discontinued at parturition, except as noted later.

Two major feeding trials were conducted, no. I in 1945 and no. II in 1946. In the former trial, the vitamin-A-supplemented cows received 1,000,000 I.U. of vitamin A daily during the 2 weeks preceding expected parturition date; in the latter, a 2-week period of supplementation (28-14 days prepartal) was added, during which time the cows received 500,000 I. U. of vitamin A daily. In pasture-supplemented groups, less concentrate was fed in trial II than in trial I in order to conserve grains. This was accomplished by shortening the period of prepartal concentrate feeding of these cows and by returning them to pasture on the fifth day postpartum instead of the fourteenth. In tables that follow, results for the eighth day, therefore, are not included for pasture groups of trial II.

Collection of samples. In order to avoid sampling difficulties, calves were not allowed to nurse. Each sample represented aliquots of well-mixed mammary products obtained by as thorough evacuation of the udder (10, 19) as possible by standard milking procedures, either hand or machine. The first milking generally was completed within 4 hours after parturition, and subsequent samples were collected at the regular morning and evening milking periods. Immediately after collection, samples were stored in the dark at 4° C. until removed for vitamin A and for carotenoid analyses, usually within 4 days.

Method of analysis. Vitamin A and carotenoids were determined by an adaptation of the Boyer *et al.* method (3). Since colostrum normally is more viscous than milk and is higher in vitamin A and carotenoids, larger volumes of ether and wash solutions were employed than prescribed in the original method.

⁴ A concentrate of natural vitamin A ester in a special soybean flour base; however, two cows received vitamin A in a liver meal base and one cow received vitamin A in the form of a fish oil concentrate.

Modifications of the procedure were as follows: For extraction, 50 and 30 ml. quantities of ether were used, respectively, in the first and the second separatory funnels. The solutions containing the non-saponifiable matter were washed by gentle mixing with 100 ml. of water, followed by shaking with 40 ml. and then 25 ml. of acidified alcoholic wash solution. The two ether solutions were combined in one separatory funnel and 15 ml. of Skellysolve B were added to decrease the water content. The combined solutions were given a final washing by shaking with 25 ml. of cold water. An all-glass assembly of about 125 ml. capacity was used for evaporation of solvents. The residue was dissolved in Skellysolve B and diluted to volumes of 25 to 50 ml., the quantity employed depending upon the concentrations of vitamin A and carotenoids. The resulting solution was used for measuring total carotenoids.⁵ A Coleman spectrophotometer was employed in the colorimetric measurements. Calibration data and a modification of the instrument for vitamin A analysis have been published (17). The antimony trichloride reagent was prepared essentially as outlined by Koehn and Sherman (13).

It was necessary to modify the procedure for colostrums of a high specific gravity since apparently either saponification or extraction was incomplete. Vitamin A and carotenoid values of colostrum samples having a specific gravity greater than 1.060 sometimes were increased as much as 25 per cent when saponification by boiling under reflux for 20 minutes was substituted for the original 3-hour saponification at room temperature.

Expression of results. The results are presented primarily as concentrations of vitamin A and of carotenoids per unit of fluid, since this yields information on both nutritive value and effects of dietary vitamin A and carotenoids on changes of each of these constituents in the mammary products. Total vitamin A and total carotenoid outputs during the first eight or nine milkings of the colostrie period also are presented. Exceptions are made to the foregoing methods of stating results and other values are discussed only when expression in different units alters interpretations.

RESULTS AND DISCUSSION

Variations among cows of the same groups. Marked individual differences were found in colostrum from cows of the same breed, lactation and dietary groups. In comparisons of concentrations of either vitamin A or carotenoids in the first samples of colostrum from cows within each of the individual experimental groups (designated in tables 2 and 3), one-third of the ratios of the highest concentration to the lowest was more than 3:1, but similar comparisons of milk near the end of the transition period revealed that only one-tenth of the corresponding ratios was more than 3:1. In colostrum of the first milking, the widest ratio (12:1) for carotenoids was found in samples from first-lactation Jerseys receiving vitamin A supplements; the widest ratio (52:1) for vitamin A was in samples from first-lactation Jerseys fed the winter barn ration. The lowest

⁵ A β -carotene calibration curve was used. Approximately 90 to 95 per cent of the total carotenoids may be considered as carotene (4, 18, 24).

value in the latter group was obtained from a heifer, J-367, that secreted an atypical colostrum that somewhat resembled whey (table 1). The extremely low vitamin A level associated with colostrum of low specific gravity was observed in secretions from only two other animals, also first-lactation heifers. Vitamin A and carotenoid levels in the milk at the end of the transition period were in the normal range.

It is difficult to account for the individual variations in colostrum, which phenomenon also has been noted by others (4, 8, 10, 11, 26, 28). Among the factors that might be involved are heredity, endocrine activity, appetite and dietary history.

Effect of breed. Although considerable variation within breeds was observed, the data from trials I and II (tables 2 and 3), especially the latter, show that con-

TABLE 1
Comparison of vitamin A and carotenoid contents of typical and atypical colostrum from Jersey heifers fed winter barn rations

Mammary secretion	Constituents	Number of milking								
		1	2	3	4	5	6 + 7 ^c	8 + 9	12 + 13	16 + 17
(μg./100 ml.)										
“Normal” ^a (Av. of 3 heifers)	Carotenoids	147	189	107	59	41	35	27	16	17
	Vitamin A	319	398	218	125	72	75	67	30	25
“Low specific gravity” ^b (Heifer J-367)	Carotenoids	19	32	32	23	31	28	23	20	22
	Vitamin A	10	26	37	40	53	54	48	36	26

^a In first colostrum, specific gravity = 1.060, total solids = 23.8 per cent and fat = 5.45 per cent; in composite of 16th and 17th milkings, corresponding values were 1.033, 13.9 and 4.27.

^b In first colostrum, specific gravity = 1.027, total solids = 9.0 per cent and fat = 1.15 per cent; in composite of 16th and 17th milkings, corresponding values were 1.033, 13.4 and 4.1.

^c Composite sample.

centrations of carotenoids in colostrum and in early milk from Jerseys and Guernseys tended to be higher than in similar secretions from Holsteins and Ayrshires. Highest values were noted most frequently in colostrum from Guernseys. The relationship of breed to vitamin A concentrations was variable and indefinite. Data reported by Sutton *et al.* (28, 29) also indicated that carotene was higher in colostrum from Guernseys than from other breeds, but the results from first- and later-lactation cows were not segregated.

Average total output of carotenoids during the transition period (first eight or nine milkings, tables 2 and 3) by Jerseys and Guernseys were higher than those from Holsteins and Ayrshires of the same experimental groups; however, in trial II there was a tendency for the latter breeds to secrete more vitamin A.

Effect of number of lactations. Vitamin A levels were higher in nine-tenths of the colostrum samples (first five milkings) from first-lactation cows than in those from cows in later lactations receiving the same treatment (tables 2 and 3). In more than one-half of the samples from the former groups, averages were at

TABLE 2

Effect of type of ration, number of lactation and breed of cows on vitamin A and on carotenoid contents of colostrum and early milk (Trial I)

Rations ^a	Breeds of cows	No. of cows of each breed	Lactation no.	Carotenoids, µg./100 ml.										Total output first 9 milkings		mg. carotenoids
				1	2	3	4	5	6 + 7 ^b	8 + 9	12 + 13	16 + 17	24 + 25			
Barn	Hol.-Ayr.	1-2	First	92	157	81	47	30	32	19	23	9	8	29		
	Jersey	4	First	115	150	88	50	38	33	26	17	19	23	31		
	Jersey	1	Later	68	38	39	42	33	38	38	33	21	21	22		
Pasture	Hol.-Ayr.	2-2	First	191	147	80	61	48	45	29 ^c	17 ^c	18	23 ^c	40 ^c		
	Holstein	1	Later	560	110	58										
	Jersey	4	First	434	351	207 ^d	152 ^c	138 ^c	96 ^c	69 ^d	39 ^c	29 ^c	36 ^c	78 ^c		
Vitamin A supplement	Jersey	1	Later	173	151	125	100	104	101	80	62	55	44	50		
	Holstein	1	First	160	85	32	25	21	13	13	5	5	6	22		
	Ayrshire	2	Later	95	69	59	32	24	26	14	19	12	16	27		
Barn	Jersey	1	Later	82	331	153	43	21	25	17	13	18	10	35		
	Hol.-Ayr.	1-2	First	324	460	183	104	71	61	32	28	24	17	72		
	Jersey	4	First	242	305	173	104	68	70	62	32	26	27	64		
Pasture	Jersey	1	Later	45	28	25	42	29	30	43	13	10	18	19		
	Hol.-Ayr.	2-2	First	416	353	171	115	54	54	43 ^c	19 ^c	18	20 ^c	83 ^c		
	Holstein	1	Later	563	101	67										
Vitamin A supplement	Jersey	4	First	436	379	200 ^d	187 ^c	157 ^c	97 ^c	65 ^d	37 ^c	31 ^c	36 ^c	87 ^c		
	Jersey	1	Later	52	62	60	73	79	76	89	60	54	33	36		
	Holstein	1	First	1360	725	338	249	199	99	42	42	54	34	177		
Vitamin A supplement	Ayrshire	2	Later	359	382	275	149	136	129	72	56	52	45	124		
	Jersey	1	Later	279	1480	700	220	100	130	76	49	42	44	165		

^a See text for description of rations.^b Composite sample.^c Two to four lactations.^d Mean calculated from one less than number of animals indicated.^e Mean calculated from two less than number of animals indicated.

TABLE 3
Effect of type of ration, number of lactation and breed of cows on vitamin A and on carotenoid contents of colostrum and early milk (Trial II)

Rations ^a	Breeds of cows	No. of cows of each breed	Lactation no.	Number of milking						Total output first 8 milkings		
				1	2	3	4	5	6		7 + 8 ^b	15 + 16
Carotenoids, µg./100 ml.												
Barn	Hol.-Ayr.	2-2	First	91	65	50	41	35	26	16	13	15
	Hol.-Ayr.	4-4	Later	78	75	39	29	26	22	18	8	20
	Jer.-Guer.	2-4	First	230	196	100	73	59	41 ^d	39	25	31 ^d
	Jersey	2	Later	199	155	51	46	36	45	40	9 ^d	27
Pasture	Hol.-Ayr.	2-2	First	185	198	99	90	60	59	42	32	32
	Hol.-Ayr.	4-3	Later	222	186	121	93	79 ^d	72 ^d	45	61 ^d	
	Jer.-Guer.	2-1	First	298	328	262	146	85	97	86	58	
	Jer.-Guer.	5-2	Later	342	341	207	179 ^d	127 ^e	138 ^e	83 ^e	92 ^f	
Vitamin A Supplement	Hol.-Ayr.	1-2	First	64	61	38	28	20	15	15	9	13
	Hol.-Ayr.	3-1	Later	100	46	23	32	23	21 ^d	19	11	23 ^d
	Jer.-Guer.	2-1	First	162	121	73	82	52	62	37	16	26
	Jer.-Guer.	1-1	Later	270	64 ^d	55	37	38	26	29	25	30
Vitamin A, µg./100 ml.												
Barn	Hol.-Ayr.	2-2	First	409	316	195	142	95	53	35	20	50
	Hol.-Ayr.	4-4	Later	148	157	79	54	48	40	34	22	41
	Jer.-Guer.	2-4	First	333	278	158	99	78	45 ^d	36	18	38 ^d
	Jersey	2	Later	276	209	43	45	45	55	42	23 ^d	33
Pasture	Hol.-Ayr.	2-2	First	443	400	182	151	86	83	65	62	
	Hol.-Ayr.	4-3	Later	190	168	98	79	80 ^d	61 ^d	38	51 ^d	
	Jer.-Guer.	2-1	First	294	317	222	125	69	73	62	49	
	Jer.-Guer.	5-2	Later	211	214	117	99 ^d	73 ^e	79 ^e	49 ^e	48 ^f	
Vitamin A Supplement	Hol.-Ayr.	1-2	First	670	725	391	227	162	103	100	43	122
	Hol.-Ayr.	3-1	Later	482	266	159	141	127	87 ^d	69	27	97 ^d
	Jer.-Guer.	2-1	First	548	387	242	270	163	180	106	40	96
	Jer.-Guer.	1-1	Later	588	169 ^d	145	100	119	83	61	45	75

^a See text for description of rations.

^b Composite sample.

^c Two to seven lactations.

^d Mean calculated from one less than number of animals indicated.

^e Mean calculated from two less than number of animals indicated.

^f Only three cows represented in mean value.

least twice as high as were those of samples from the latter groups. On the other hand, only about two-thirds of the colostrum samples from first-lactation cows had a higher average carotenoid concentration than did those from later-lactation cows, and in many cases there was not a consistent trend. Although calculations of carotenoids per unit of fat revealed higher values for first-lactation cows more frequently than did calculations per unit of secretion, differences were not marked enough to justify definite conclusions.

A further comparison of carotenoid and vitamin A contents of early mammary secretions from two different lactation groups is shown in table 4. As also noted

TABLE 4

Effect of the number of lactation on the carotenoid and the vitamin A contents of colostrum and early milk. (Same cows on similar dietary regimens used for comparison)

Vitamin A constituents	No. of Lactations	No.	Number of milking							
			1	2	3	4	5	6	7 + 8 ^a	15 + 16
Winter barn ration										
<i>μg./100 ml.</i>										
Carotenoids	4	1st	121	179	88	49	35	35	23	10 ^b
		2nd	136	111	44	39	30	33	27	10 ^b
Vitamin A	4	1st	416	551	233	125	80	69	37	20 ^b
		2nd	196	160	56	46	48	49	35	25 ^b
Pasture										
<i>μg./100 ml.</i>										
Carotenoids	3	1st	221	188	109	80	82 ^b	42 ^b	36 ^b	29 ^c
		2nd	184	185	157	116	73 ^b	74 ^b	42 ^b	8 ^c
Vitamin A	3	1st	316	253	152	108	66 ^b	42 ^b	47 ^b	10 ^c
		2nd	127	134	107	81	63 ^b	51 ^b	43 ^b	30 ^c

^a Composite sample.

^b Average calculated from one less than number of animals indicated.

^c Figure represents value from one cow only.

by others (4, 10, 11), carotenoid concentrations of colostrum were not related definitely to number of lactation, but vitamin A values were consistently higher in samples from the cows during their first lactation. These differences in vitamin A possibly are related to length of the non-lactating period before calving (26).

Relative effects of type of ration fed during latter stages of gestation. a. Roughages (carotenoids). It is shown by data of tables 2 and 3 that carotenoid contents of colostrum and early milk from pasture-supplemented cows of the same breed and lactation groups were from two to four times higher than corresponding mammary secretions from cows maintained on typical barn rations. An exception, however, was noted for the first-lactation Holstein-Ayrshire group of trial I in which such marked differences were not found in samples from the second through the thirteenth milkings.

Comparisons of the effects of pasture and of barn rations on vitamin A levels of the mammary secretions (tables 2 and 3) showed that differences in the averages generally were less marked than noted for carotenoids, vitamin A concentrations being higher for the pasture groups in only two-thirds of the samples compared. Supplementary calculations^a indicated that vitamin A concentrations per unit of fat in colostrum and early milk from Jerseys and Guernseys of the pasture groups were no higher than those found in samples from cows of the same breeds fed only barn rations. Total output of Vitamin A, however, was consistently higher in colostrum from cows on pasture than from those receiving only barn rations.

If data on vitamin A and carotenoids were combined as total vitamin A potency, average values for pasture cows would be appreciably higher than those for similar groups of barn-ration cows in more than three-fourths of the samples.

Additional information on the comparative effects of dry rations and of pasture upon vitamin A and carotenoids of colostrum and early milk was obtained from a study involving five cows (Holsteins and Ayrshires) that were fed a carotenoid-low basal ration in which beet pulp replaced a part of the roughages. Three of the cows were allowed supplemental pasture both pre- and postpartally. Sampling and treatment of colostrum and milk were as previously described, except only secretions from the left half of the udder were used. Some samples were frozen and stored, which probably had little or no effect on vitamin A and carotenoid values (10). Concentrations of both vitamin A and carotenoids were more than two times higher in colostrum and early milk from cows receiving pasture than from those receiving only low-carotenoid rations.

These results are in accord with earlier studies (14, 21) in which increases of total vitamin A potency were observed, but are somewhat discordant with those of others (25) in which carotene supplements increased only carotene of colostic fat. The increases in carotenoids resulting from pasture grazing are in harmony with observations of Henry *et al.* (11), but elevation of the levels of vitamin A, as reported herein, were not indicated by the latter authors. Failure of Stewart and McCallum (27) to detect increases of carotene in colostrum from cows receiving prepartal supplements of carrots might have been due either to amounts ingested and/or to sampling methods employed.

b. Vitamin A supplements. Colostrum and early milk from cows receiving vitamin A supplements prepartally contained appreciably more vitamin A than did the secretions from cows fed only barn rations (tables 2 and 3). In more than half of the samples representing each of the first six milkings, average vitamin A concentrations in the secretions from cows receiving supplemental vitamin A were at least twice as high as were those from similar cows fed only barn rations. Unexpectedly, some of the higher vitamin A levels were found in colostrum from cows fed vitamin A supplement for the shorter periods of time. Also, cows receiving vitamin A produced colostrum of a higher vitamin A content than did cows receiving pasture, but, in general, differences were less marked than those found when vitamin A-supplemented and barn-ration cows were compared.

^a Space does not permit presentation of these data as well as some other in later sections; data are available to anyone interested.

Increases in vitamin A levels of colostrum following supplementation of the ration with this vitamin also have been noted by others (7, 25, 30). Stewart and McCallum (27), however, did not find cod-liver oil supplements providing 70,000 I. U. vitamin A per cow daily effective in increasing the vitamin A levels of colostrum. This low level of supplementation, the sampling methods and the possible reduction of milk fat by cod-liver oil (2) might have affected their results.

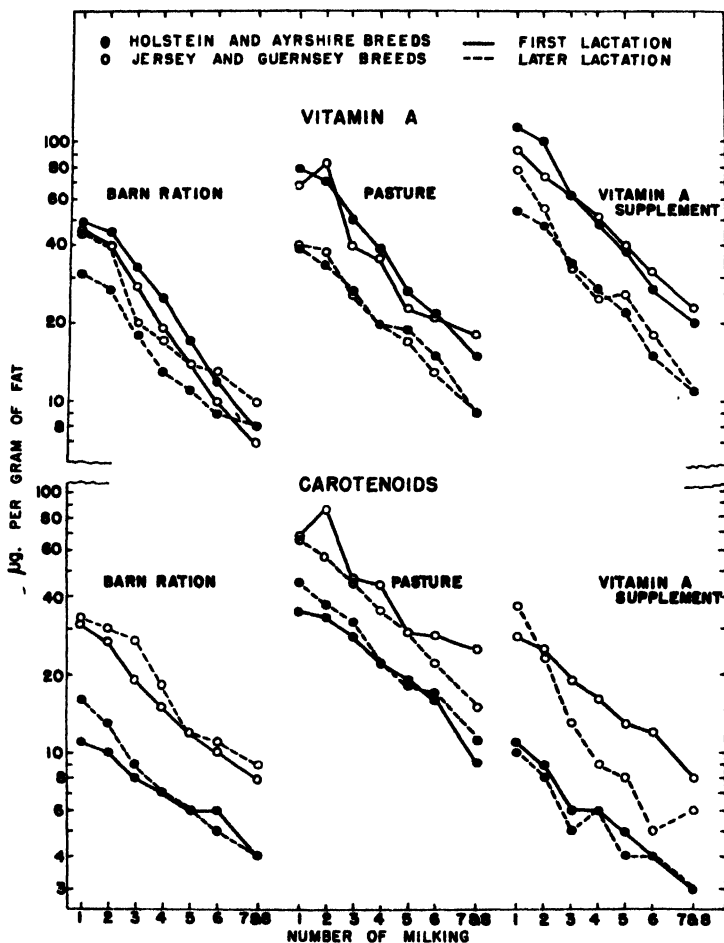


FIG. 1. Changes in concentration of vitamin A and of carotenoids in colostric fat during the transition period.

Several reports previously reviewed (32) indicated that a large intake of vitamin A decreases the carotenoid content of milk. When the diet of cows that had various degrees of mastitis was supplemented with 1,250,000 I. U. of vitamin A daily, both pre- and postpartally, suppression of carotenoids in colostrum and early milk seemed to occur, as also noted by Esh *et al.* (7). On the other hand,

when supplementation with 1,000,000 I. U. of vitamin A daily was discontinued at parturition, the effect on carotenoid contents of the secretions was variable (tables 2 and 3). In the present study, suppression of carotenoids was observed somewhat more frequently in the colostric fat than in the complete mammary secretion; explanation for this difference is obscure.

Effect of stage in the transition period. Vitamin A and carotenoid contents of the mammary secretions normally decreased rapidly during the first few milkings following parturition (tables 2, 3, and 4), as also observed by other investigators. Characteristic transitional changes of vitamin A and carotenoids in colostric fat (trial II) are presented on a semi-logarithmic scale in figure 1. In spite of irregularities observed, especially in secretions from some of the smaller groups of cows, decreases of both vitamin A and carotenoids tended to follow a logarithmic course. This trend is more pronounced when results are expressed as content per unit of fat instead of per unit of fluid secretion. Rates of change resemble those found for tocopherols (19), and a similar trend also has been reported for carotene (22). Observations over extended periods disclosed that the rates of change in concentrations of vitamin A and carotenoids generally had undergone a marked decrease by the eighth to the tenth milking postpartum. The rates of change of vitamin A and carotenoids of colostric fat apparently were not affected markedly by breed, number of lactation and type of prepartal ration.

However, some exceptions to the general trend were noted. These occurred especially in the group averages of samples from first-lactation, pasture-supplemented Jerseys and Guernseys of trial II (fig. 1) and in those from the individual Jersey cows of the barn and the pasture groups of trial I (table 2). These apparently anomalous tendencies might have been due to changes that were observed in the fat content of the secretions. It is not known to what extent results were affected by inability to evacuate the gland completely at each milking (29).

In transitional mammary secretions from three cows receiving rations fortified with vitamin A, 1,250,000 I. U. daily (32), both pre- and postpartally the concentrations of this vitamin remained high over a longer period than did the levels in corresponding secretions from comparable cows (trials I and II) receiving 1,000,000 I. U. of supplemental vitamin A daily only to the time of parturition. The differences were marked after the third milking. At the end of the transition, the values of vitamin A in the milk of the postpartally supplemented cows were eight times higher than in milk from cows that were not supplemented after parturition; the concentrations in the fat were three times higher.

SUMMARY

A study was made of the effects of individuality, breed, lactation number and prepartal diets on vitamin A and carotenoid contents of colostrum and transitional milk from 86 cows representing four dairy breeds.

Marked individual differences were found in the vitamin A potency of colostrum from cows of the same breed, lactation (first or later) and dietary group. A greater degree of variability was observed in early colostrum than in the milk

from the same cows at the end of the transition period. Occasionally, first-lactation cows secreted an atypical colostrum in which vitamin A and carotenoids were abnormally low.

The carotenoid content of colostrum and early milk from Jerseys and Guernseys was higher than in the corresponding secretions from Holsteins and Ayrshires, but differences with respect to Vitamin A were not marked.

Concentrations of vitamin A in the mammary secretions from first-lactation cows generally were higher than in those from cows in later lactations, but consistent differences in carotenoids were not observed.

Access to pasture during the terminal weeks of gestation produced higher levels of carotenoids in colostrum and transitional milk than did typical barn rations. Although levels of vitamin A generally were increased by pasture grazing, the increase was not so great as observed for carotenoids.

High intakes of vitamin A concentrates (500,000 and 1,000,000 I. U. daily, respectively, 4-2 and 2-0 weeks prepartal increased the vitamin A content of colostrum and transitional milk to levels higher than those in corresponding mammary products from cows fed either typical barn rations or pasture.

Supplementation of the ration with 1,250,000 I. U. of vitamin A daily both pre- and postpartally, tended to decrease the concentration of carotenoids in the mammary secretions; but supplementation during the prepartal period only (4-2 and 2-0 weeks, at levels of 500,000 and 1,000,000 I. U. daily, respectively) had no consistent effect on the carotenoids.

Concentrations of vitamin A and carotenoids in first colostrum generally were several times higher than in milk at the end of the transition period. Even when daily supplementation with high levels of vitamin A was continued postpartally, as well as during the prepartal period, the first colostrum averaged almost 3 times higher in vitamin A content than did milk from the same cows 14 days later.

The transition in vitamin A and in carotenoid contents of colostrie fat was rapid during the first eight milkings, both constituents following a similar logarithmic trend. Neither number of lactation (first or later), breed, nor prepartal rations seemed to influence appreciably the rate of transition, but in all cases definitely smaller rates of change were evident by the eighth to the tenth milking. Since vitamin A and carotenoids of colostrum are concentrated primarily in the fat, the logarithmic trend usually was manifested more clearly when results were expressed as concentrations per unit of colostrie fat instead of as concentrations per unit of total secretion.

ACKNOWLEDGMENTS

The authors wish to thank Distillation Products, Inc., Rochester, N. Y., World Products, Inc., New York, N. Y., and the Borden Company, New York, N. Y., for supplying the vitamin concentrates. The authors are grateful to Kenneth Brooks and William Mudge for assistance in management of the experimental animals, and to Mrs. Helen Hamlin for assistance in summarization of data.

REFERENCES

- (1) BARRON, N. S. Vitamin A and Its Relationship to Some of the Commoner Diseases of Farm Animals. *Vet. Record*, 54: 29-37. 1942.
- (2) BLAXTER, K. L., KON, S. K., AND THOMPSON, S. Y. The Effect of Feeding Sharkliver Oil to Cows on the Yield and Composition, and on the Vitamin A and Carotene Content of the Milk. *J. Dairy Research*, 14: 225-230. 1946.
- (3) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. *Ind. Eng. Chem., Anal. Ed.*, 16: 101-102. 1944.
- (4) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. II. The Carotene and Vitamin A Content of Cows' Colostrum. *Biochem. J.*, 27: 1998-2005. 1933.
- (5) DANN, W. J. The Transmission of Vitamin A from Parents to Young in Mammals. V. The Vitamin A and Carotenoid Contents of Human Colostrum and Milk. *Biochem. J.*, 30: 1644-1651. 1936.
- (6) DRUMMOND, J. C., COWARD, K. H., AND WATSON, A. F. Researches on Vitamin A. VII. Notes on the Factors Influencing the Value of Milk and Butter as Sources of Vitamin A. *Biochem. J.*, 15: 540-552. 1921.
- (7) ESH, G. C., SUTTON, T. S., HIBBS, J. W. AND KRAUSS, W. E. The Effect of Soya-Phosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, 31: 461-478. 1948.
- (8) FRENCH, M. H. The Carotene and Vitamin A Values of Colostrum from High-Grade Cows. *E. African Agr. J.*, 8: 174-175. 1943.
- (9) GILLAM, A. E., HEILBRON, I. M., FERGUSON, W. S., AND WATSON, S. J. Variations in the Carotene and Vitamin A Values of the Milk Fat (Butter) of Cattle of Typical English Breeds. *Biochem. J.*, 30: 1728-34. 1936.
- (10) HANSEN, R. G., PHILLIPS, P. H., AND SMITH, V. R. Colostrum Milk and Its Vitamin A Content. *J. Dairy Sci.*, 29: 809-814. 1946.
- (11) HENRY, K. M., HOUSTON, J., AND KON, S. K. The Vitamin A and Carotene Contents of Shorthorn Colostrum. *J. Dairy Research*, 11: 1-8. 1940.
- (12) HRUBERTZ, M. C., DEUEL, H. J. JR., AND HANLEY, B. J. Studies on Carotenoid Metabolism. V. The Effect of a High Vitamin A Intake on the Composition of Human Milk. *J. Nutrition*, 29: 245-254. 1945.
- (13) KOHN, C. J., AND SHERMAN, W. C. The Determination of Vitamin A and Carotene with the Photoelectric Colorimeter. *J. Biol. Chem.*, 132: 527-538. 1940.
- (14) KRAMER, M. M., BAIR, M. D., KUNERTH, B. L., AND RIDDELL, W. H. The Vitamin A Value of Colostrum and Milk of Four Cows Determined by the Single-Feeding Method. *J. Agr. Research*, 56: 227-232. 1938.
- (15) LESHER, M., BRODY, J. K., WILLIAMS, H. H., AND MACY, I. G. Human Milk Studies. XXVI. Vitamin A and Carotenoid Contents of Colostrum and Mature Human Milk. *Am. J. Diseases Children*, 70: 182-192. 1945.
- (16) MEULEMANS, O., AND DE HAAS, J. H. The Carotene and Vitamin A Contents of Human Milk of Batavia (Dutch East Indies). *Geneesk. Tijdschr. Nederland. Indië*, 76: 1538-1571. 1936. (Chem. Abs., 30: 7153. 1936).
- (17) PARRISH, D. B., MARTIN, W. H., ATKESON, F. W., AND HUGHES, J. S. The Vitamin A and Carotene Content of Market Butter Produced in Kansas. *J. Dairy Sci.*, 29: 91-99. 1946.
- (18) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S. The State of Vitamin A in Colostrum and in Milk. *J. Biol. Chem.*, 167: 673-678. 1947.
- (19) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S. Properties of the Colostrum of the Dairy Cow. I. Tocopherol Levels in the Colostrum and in the Early Milk. *J. Dairy Sci.*, 30: 849-860. 1947.
- (20) RAY SARKAR, B. C. Carotene and Vitamin A in the Colostrum of Cows of Typical Indian Breeds. *J. Dairy Sci.*, 31: 479-487. 1948.

- (21) REED, O. E. Report of the Chief of the Bureau of Dairy Industry. Pp. 14-15. 1944.
- (22) RICHTER, F. Der Fätslosliche Farbstoff des Kuh-Kolostrums and sein Verhalten während der Kolostral-periode. *Kühn-Arch.*, 49: 269-290. 1938.
- (23) SATTERFIELD, G. H., CLEGG, R. E., AND HOLMES, A. D. Vitamin A Content of Sheep Colostrum and Milk. *Food Research*, 9: 206-211. 1944.
- (24) SEMB, J., BAUMANN, C. A., AND STEENBOCK, H. Fat-Soluble Vitamins. XLI. The Carotene and Vitamin A Contents of Colostrum. *J. Biol. Chem.*, 107: 697-703. 1934.
- (25) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., WHITING, F., NORTON, C. L., AND TURK, K. L. The Relationship of the Prepartum Diet to the Carotene and Vitamin A Content of Bovine Colostrum. *J. Dairy Sci.*, 30: 343-350. 1947.
- (26) STEWART, J., AND MCCALLUM, J. W. The Vitamin A Content of the Colostrum of Dairy Cows. *J. Agr. Sci.*, 28: 428-436. 1938.
- (27) STEWART, J., AND MCCALLUM, J. W. The Effect of a Vitamin A Rich Diet on the Vitamin A Content of the Colostrum of Dairy Cows. *J. Dairy Research*, 13: 1-4. 1942.
- (28) SUTTON, T. S., KAESER, H. E., AND SOLDNER, P. A. Changes in the Level of Vitamin A and Carotene in the Blood Plasma of Dairy Cows Associated with Parturition and Beginning Lactation. *J. Dairy Sci.*, 28: 933-939. 1945.
- (29) SUTTON, T. S., WAENER, R. G., AND KAESER, H. E. The Concentration and Output of Carotenoid Pigments, Vitamin A, and Riboflavin in the Colostrum and Milk of Dairy Cows. *J. Dairy Sci.*, 30: 927-932. 1947.
- (30) THOMAS, J. W., LOOSLI, J. K., AND WILLIAM, J. P. Placental and Mammary Transfer of Vitamin A in Swine and Goats as Affected by the Prepartum Diet. *J. Animal Sci.*, 6: 141-145. 1947.
- (31) UNDERWOOD, E. J. AND CURNOW, D. H. Vitamin A in the Nutrition of Sheep in Western Australia. IV. The Vitamin A Content of Ewe's Milk and Colostrum. *Australian Vet. J.*, 20: 282-285. 1944.
- (32) WISE, G. H., ATKESON, F. W., CALDWELL, M. J., PARRISH, D. B., AND HUGHES, J. S. Effects of High Vitamin A Intake on Milk and Fat Yields and on Vitamin A Constituents in Milk, Blood, and Livers of Dairy Cows. *J. Dairy Sci.*, 30: 279-291. 1947.

THE ISOLATION OF FURFURYL ALCOHOL FROM HEATED SKIMMILK

STUART PATTON¹ AND DONALD V. JOSEPHSON¹

Ohio State University, Department of Dairy Technology

The identification of some of the end products resulting from the heating of skimmilk should present a workable approach to the problems of browning and caramelized flavor development in milk and other dairy products. The constituents native to milk are well known and with some knowledge of the end products, formulation and comprehension of the underlying chemical mechanisms would be facilitated greatly. Very little work has been done in this field of dairy research. Most of the information pertaining to the subject concerns the formation of volatile acids (formic in particular) and lactic acid. Acid production in milk as a result of heating has been observed by many workers; the findings of Gould (5, 6) and Gould and Frantz (7) are most significant on this point. Hankinson *et al.* (8) have attempted to identify a dialyzable substance from raw milk which when heated gives rise to a heated milk flavor and odor. Even though their efforts to isolate this compound were unsuccessful, they did succeed in establishing some of the characteristics of the compound.

Of late, considerable interest has attached to the possible role of furan compounds in the browning of many stored foods. Although it might be expected that such compounds are generated in milk under the influence of heat, there seems to be no direct evidence in the literature supporting this theory.

In view of the scarcity of information on this subject and the obvious value which further findings would have, it seemed worthwhile to attempt isolation and characterization of compounds formed in milk by heat.

EXPERIMENTAL

Removal of ether-soluble substances from heated skimmilk. Skimmilk pasteurized at 62.2° C. for 30 minutes was placed in three 2-gallon milk cans and autoclaved, 5 gallons at a time, for 90 minutes at 126.6° C. The autoclaved milk was allowed to cool over night in a refrigerator at 4° C. The following day the milk was extracted with an equal volume of redistilled ethyl ether.

The extraction was done by hand using either a 2-liter or 4-liter aspirator bottle with outlets at the bottom and top for separating the milk and ether layers. One- or 2-quart quantities of milk were extracted at a time, and the extraction was accomplished by vigorous shaking of the ether-milk mixture for a period of 1 to two minutes. The ether-soluble substances were concentrated by distilling off the excess ether. This ether subsequently was reused for further extraction. All stoppers were lined with tinfoil to prevent the accumulation of impurities in the ether. Two separate large scale experiments were carried out involving the extraction of 22 gallons of heated milk in the first and 25 gallons in the second.

Received for publication September 13, 1948.

¹ Present address: Department of Dairy Husbandry, The Pennsylvania State College, State College, Pa.

Purification of the ether-soluble substances. When the ether extract was concentrated to a volume of approximately 200 ml., the balance of the ether was removed by vacuum, using a water pump. Following removal of the ether, the residue immediately was taken up in 300 ml. of water. The water solution, which also contained insoluble matter, was extracted with two 50 ml. portions of petroleum ether (35–65° C. boiling fraction) to remove the true petroleum ether-soluble substances. The water fraction then was extracted with three 600 ml. volumes of redistilled ethyl ether. The ether solution was dried by shaking with anhydrous sodium sulfate and concentrated again in the manner outlined previously for the crude ether extract. In this way, it was possible to separate the true ether-soluble substances from those predominantly water-soluble or petroleum ether-soluble.

Distillation of the ether-soluble residuc. The dry ether-soluble residue (approximately 10 ml. of material) was transferred to a 25 ml. distilling flask which then was fitted with a capillary and a 100° C. thermometer. This flask delivered into a 10 ml. distilling flask. The side arm of the receiving flask was connected to a vacuum system which operated at 1 to 2 mm. pressure in the first experiment and at less than 1 mm. in the second. A warm water bath was used as the heating medium and the receiving flask was submerged in an ice bath.

In the case of the first experiment, 1 ml. of material distilled between 22 and 57° C., but at 57° C. the temperature held quite steadily. A new receiving flask was inserted and an additional 2 to 3 ml. distilled between 57 and 59° C. At this point, the water bath temperature was 65° C. Raising the temperature of the bath to 100° C. accomplished no further distillation and merely darkened the residue in the distilling flask so the distillation was halted at this point. The fraction boiling between 57 and 59° C. was redistilled under vacuum, the first few drops being discarded. The same procedure was used in the second experiment and essentially the same results were obtained, except that the major portion of the residue distilled between 46 and 48° C., no doubt due to the higher vacuum. The procedure used in the isolation of this distillate from the autoclaved skimmilk is illustrated schematically in figure 1.

Characteristics of the compound constituting the vacuum distillate. Since the vacuum distillate showed some indication of purity by its constant boiling characteristic, it was thought advisable to attempt identification of the major compound present. The first experiment revealed the following properties of the distillate: Boiling point—740 mm., 165° C.; refractive index—(n_D^{25}), 1.485; density—(D_4^{25}), 1.11; freezing point—, < -30° C.; water soluble, ether soluble; containing no nitrogen, sulfur or halogen; average carbon 60.15 per cent; average hydrogen 6.07 per cent.

The carbon-hydrogen analysis of the distillate corresponded well to an empirical formula of $C_5H_6O_2$. It was observed further that the distillate gave typical reactions for certain furan compounds. It turned a pine splint moistened with concentrated hydrochloric acid blue-green (9). It reddened aniline-hydrochloric acid reagent (10). It also produced a black resin with concentrated sulphuric acid.

At this point it was suspected that the distillate might be mainly furfuryl alcohol and a number of tests were performed with the distillate and a control sample of furfuryl alcohol. The results indicated great similarity in respect to

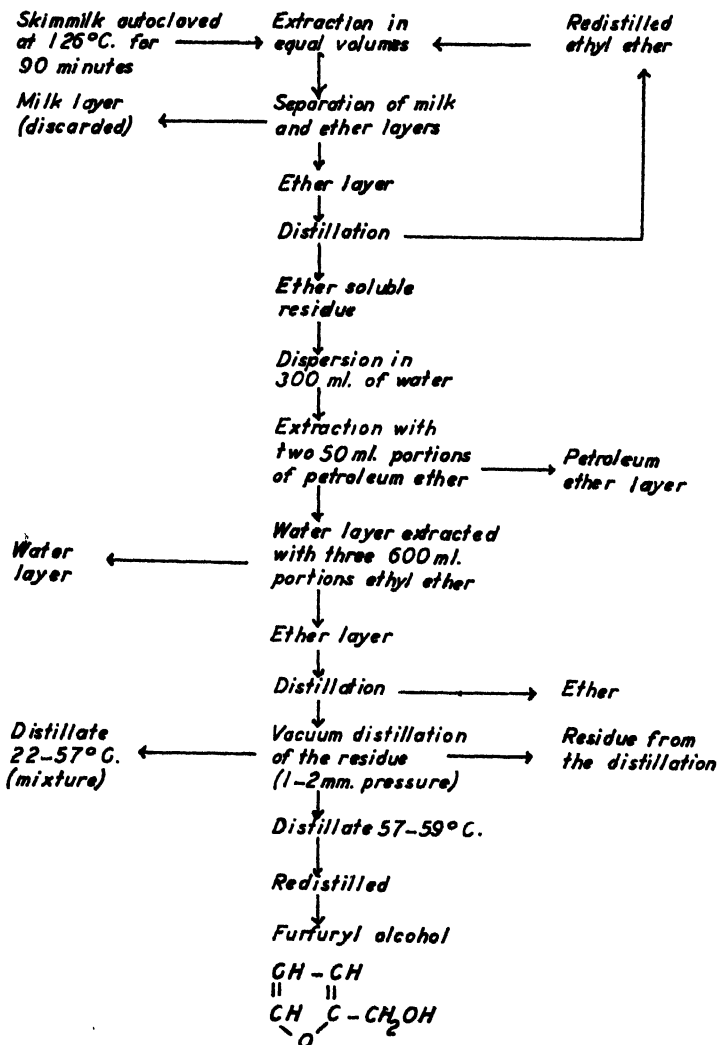


Fig. 1. Flow diagram for the isolation of furfuryl alcohol from heated skimmilk.

bromine uptake, reduction of hot ammoniacal silver nitrate and alkaline permanganate, inertness toward Fehling's reagent and toward 2,4-dinitrophenylhydrazine reagent.

Preparation of derivatives. In order to obtain more material for the prepa-

ration of suitable derivatives and to verify the findings of the first experiment, it was necessary to conduct a second experiment. Investigation of the distillate from the second trial revealed it to have the same physical and chemical properties as indicated for that of the first.

The naphthyl and phenyl urethanes and the 3,5-dinitrobenzoate derivatives of the distillate were prepared in accordance with the procedures outlined by McElvain (11) and Shriner and Fuson (14). Table 1 gives the melting points of the derivatives obtained together with those given by Huntress and Mulliken (9) for furfuryl alcohol. These data indicate quite conclusively that the distillate is mainly furfuryl alcohol.

Procedure and observation in connection with the control experiment. In order to establish the fact that in this investigation furfuryl alcohol was produced in skimmilk by heating and was not a constituent native to the milk or an accumulated impurity resulting from the method or reagents used, it seemed ad-

TABLE 1

Melting points of derivatives prepared from the vacuum distillate obtained by ether extraction of heated skimmilk as compared with those for the same known derivatives of furfuryl alcohol.

Derivatives	Observed melting point (°C.)	Melting point of furfuryl alcohol derivatives ^a (°C.)
Naphthyl urethane	129-130	129-130
Phenyl urethane	43-45	45
3,5-dinitrobenzoate	79-81	80-81

^a Huntress and Mulliken (9).

visable to conduct a control experiment. Five gallons of fresh raw skimmilk were extracted with ether and the ether extract concentrated in the same manner as described for the heated skimmilk. Qualitative tests for furan compounds were performed on the ether extract residue. The pine splint and aniline-hydrochloric acid tests were negative. The residue did not darken sulfuric acid appreciably. The results of these tests are sufficient evidence that furfuryl alcohol is not a normal constituent of raw milk but is produced by heat treatment.

DISCUSSION

It has been known for some time that furfural may be produced by heating pentosans in the presence of concentrated sulfuric acid. The general character of the reaction appears to be the removal of three molecules of water from the pentose molecule, although the reaction may be stepwise and somewhat more complicated (3). This method serves as the basis for the commercial production of furfural, which is a very useful intermediate in the synthesis of many other furan derivatives (1, 13). Furan compounds also have been synthesized in the laboratory by ring closure of certain diketones and other suitable compounds (3). Whereas pentoses yield furfural when heated in an acid medium, hexoses yield hydroxy-methyl furfural (15).

The chemistry of furan compounds has been reviewed extensively by Gilman and Wright (4) with respect to syntheses, reactions, aromatic character of the ring, ring substitution and scission. Further review of the subject is not within the scope of this investigation, but it should be pointed out that the mechanism by which furfural may be oxidized to the acid or reduced to the alcohol (3) may have some bearing on the formation of furfuryl alcohol in heated milk.

The isolation of furfuryl alcohol was somewhat incidental in the present investigation, since the major objective was the isolation of heat-generated flavor compounds in milk. However, its importance as an associated substance and an end product of the chemical reactions initiated in milk by heat should not be overlooked. At the very least, its presence is an indication that furan compounds are involved in the chemical changes induced in milk by heat, and the possibility exists that furfuryl alcohol is produced from furfural under the influence of the strong reducing conditions existing in heated milk. Sulfhydryl groups which are strong reducing substances and which have been observed to disappear during the processes of browning and caramelized flavor development in milk may be implicated in these changes. For example, furfuryl mercaptan in low concentrations has an odor of roasted coffee, and several observers have described the odor of the ether extract residue in this investigation as resembling that of coffee. Although such thinking is quite speculative, the possibility that furan compounds are involved in the caramelized flavor mechanism should not be overlooked.

It would be logical to assume that furfuryl alcohol is a heat degradation product of lactose, although proof is lacking on this point. Following this assumption a step further, it is evident that the chemistry must be somewhat devious, since hexoses give rise to six carbon furans. A decarboxylation mechanism might account for this inconsistency. Another potential source of furan compounds in milk is ascorbic acid. Cranston (2) recently has shown that ascorbic acid may yield furfural under certain conditions. Patton and Josephson (12) have observed that the addition of ascorbic acid to raw milk at the rate of 1 g. per liter will bring about the development of caramelized flavor at time-temperatures much lower than those noted under normal conditions (90° C.-flash). The role of ascorbic acid under these conditions is not clear, but its possible relationship to caramelized flavor and furan compounds is a point of interest.

SUMMARY AND CONCLUSIONS

A method for removing and purifying the ethyl ether-soluble substances of heated skimmilk is presented. Using this method in combination with a vacuum distillation technique, it was possible to isolate furfuryl alcohol in a fairly high state of purity from heated skimmilk. Confirmatory evidence of the presence of furfuryl alcohol is given by the results of qualitative tests and the preparation of suitable derivatives. The control experiment demonstrated that furfuryl alcohol is a compound generated in milk by heat and is not a normal constituent of unheated milk.

Although the significance of furfuryl alcohol as an end product of the heat

induced chemical reactions in skimmilk is not entirely evident at this time, the knowledge of its presence promises to be valuable in further clarifying the nature of browning and caramelized flavor development in milk and milk products.

ACKNOWLEDGEMENT

This paper reports research undertaken by The Ohio State University Research Foundation in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 216 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

The authors are indebted to Dr. F. E. Deatherage, of the Agricultural Chemistry Department, The Ohio State University, for his helpful suggestions and interest in this research.

REFERENCES

- (1) CASS, O. W. Chemical Intermediates from Furfural. *Ind. Eng. Chem.*, **40**: 216-219. 1948.
- (2) CRANSTON, H. A. Personal Communication. 1948.
- (3) DUNLOP, A. P. Furfural Formation and Behavior. *Ind. Eng. Chem.*, **40**: 204-209. 1948.
- (4) GILMAN, H., AND WRIGHT, G. F. Nuclear Substitution and Orientation of Furan Types. *Chem. Revs.*, **11**: 323. 1932.
- (5) GOULD, I. A. Lactic Acid in Dairy Products. III. The Effect of Heat on Total Acid and Lactic Acid Production and on Lactose Destruction. *J. Dairy Sci.* **28**(5): 367-377. 1945.
- (6) GOULD, I. A. The Formation of Volatile Acids in Milk by High Temperature Heat Treatment. *J. Dairy Sci.*, **28**(5): 379-386. 1945.
- (7) GOULD, I. A., AND FRANTZ, R. S. Formic Acid Content of Milk Heated to High Temperatures as Determined by the Distillation Procedure. *J. Dairy Sci.*, **29**(1): 27-31. 1946.
- (8) HANKINSON, C. L., ELLERTSON, M. E., AND GLEIM, WM. Studies of the Effect of Heat on Milk Dialysates. *J. Dairy Sci.*, **29**(10): 727. 1946.
- (9) HUNTRESS, E. H., AND MULLIKEN, S. P. Identification of Pure Organic Compounds. Order I. John Wiley and Sons, Inc., New York. P. 463. 1941.
- (10) LEVINE, V. E., AND SEAMAN, C. L. Critical Study of Shear's Aniline-Hydrochloric Acid Reaction Associated with Vitamin D. I. Furan and Derivatives. *Biochem. J.*, **27**: 2047. 1933.
- (11) MCELVAIN, S. M. The Characterization of Organic Compounds. The Macmillan Co., New York. Pp. 193-197. 1947.
- (12) PATTON, S., AND JOSEPHSON, D. V. Unpublished data.
- (13) Quaker Oats Co. Handbook of the Furans. The Quaker Oats Co., Chemicals Dept., Chicago, Ill.
- (14) SHRINER, R. L., AND FUSON, R. C. The Systematic Identification of Organic Compounds. 3rd Ed. John Wiley and Sons, Inc., New York. Pp. 163-166. 1948.
- (15) WERTHEIM, E. Textbook of Organic Chemistry. The Blakiston Co., Philadelphia and Toronto. P. 354. 1947.

FUSED TRICALCIUM PHOSPHATE AS A LOW-FLUORINE PHOSPHORUS SUPPLEMENT FOR DAIRY CATTLE¹

R. E. MATHER,² A. D. PRATT,³ AND C. W. HOLDAWAY⁴

*Department of Dairy Husbandry, Virginia Agricultural
Experiment Station, Blacksburg*

Added impetus was given during the recent world war to the search for a phosphorus supplement for cattle which safely would replace the bone meal in the ration, due to the shortage of feeding-grade bone meal. The Tennessee Valley Authority developed a process for defluorinating rock phosphate which lowered the fluorine considerably and lowered the phosphorus only slightly (7). This process involves fusion of the rock phosphate in a shaft furnace and quenching in high-velocity water jets when the fluorine has been driven off. From an original material containing 3.5 to 4.0 per cent of fluorine, a fused tricalcium phosphate containing less than 0.4 per cent of fluorine can be produced.

REVIEW OF LITERATURE

Early attempts in the use of raw rock phosphate as a calcium and phosphorus supplement usually met with disaster, although the material seemed almost ideal because the proportions of calcium and phosphorus in the phosphate were in the same ratio as in the bones of animals. The toxic effect of this material was traced to its fluorine content.

The literature relating to fluorine in the ration of various animals has been reviewed by Mitchell (9). Work with dairy cattle has been reported by Reed and Huffman (12) and by Phillips *et al.* (11). Reed and Huffman showed that when raw rock phosphate was added to the ration at the rate of 1.5 per cent of the grain mixture, poor health, reduced appetite, reduced milk production and badly worn, cold-sensitive teeth resulted. The metatarsal bones and maxillae were exostotic and there was some evidence of ankylosis. However, reproduction did not seem to be affected. A complex mineral mixture produced similar results, probably due to the use of raw rock phosphate in the mixture.

Phillips *et al.* (11) supplied six groups of three animals each with varying amounts of minerals; three of the grain rations contained 0.022, 0.044 and 0.088 per cent fluorine from raw rock phosphate. The rate of growth to 2 years was

Received for publication September 15, 1948.

¹ Published with the approval of the Director of the Virginia Agricultural Experiment Station.

² Present address: New Jersey Agricultural Experiment Station, Sussex.

³ Present address: Ohio Agricultural Experiment Station, Wooster.

⁴ The authors wish to thank Dr. R. D. Hatch, Dr. W. L. Ingalls, and Dr. W. B. Bell of the Biology Department for their assistance in the examination of the animals in this experiment, and Prof. J. F. Eheart of the Agricultural Chemistry Department, and Mr. J. H. Walthall and Dr. R. J. Jones of the Tennessee Valley Authority for their assistance in supplying chemical data on the samples obtained in the experiment.

reduced slightly on the high fluorine rations but weight differences increased with each lactation; milk production also was markedly reduced. In addition to causing exostoses of the bones and excessive abrasion of the teeth, high fluorine intake interfered with tissue respiration through its effect on enzyme systems. The critical level of fluorine tolerance was considered to be 2-3 mg. per kg. of body weight.

The value of fused tricalcium phosphate as a phosphorus supplement has been studied with rats (1, 3, 4, 14) and with chicks (2, 5, 8). In general, the phosphorus of the fused tricalcium phosphate has been found slightly less available than in tricalcium phosphate, secondary calcium phosphate or bone meal, when the amounts fed were at the borderline in regard to sufficiency of phosphorus. In some cases, with larger amounts of phosphorus available, differences were less marked. Gullickson and Olson (6) studied a defluorinated rock phosphate produced by calcination and acid treatment with less than 0.1 per cent of fluorine. Differences in weight gains of heifers were slightly in favor of the bone meal group, but the difference was not significant.

On reviewing published work, Mitchell (9) stated: "One could not be far wrong in assuming, on the basis of available data, that a level of 0.01 per cent of fluorine in the total dry rations of these animals is approximately borderline between safe and unsafe concentrations." He referred to pigs, sheep and cattle. This statement was the basis for the levels of fluorine used in this experiment, with one group at approximately one-fourth of this amount which was considered as a safe level, one group at this 0.01 per cent level as borderline, and one group at twice this level to produce readily-observed symptoms.

EXPERIMENTAL

Six Holstein bull calves were divided into three groups of two each, at random. They were castrated between the ages of 4 and 6 weeks and were placed on experiment March 24, 1944, at which time they ranged in age from 3 to 10 weeks. Whole milk was fed up to the age of 6 weeks, when it was replaced gradually by calf starter. At the age of 5 months, the starter was replaced gradually by a simple concentrate mixture. Hay was fed *ad libitum* from the time the calves would eat it. Silage was fed during the winter months after the calves were approximately 9 months of age. The steers were not pastured at any time.

The calf starter was a commercial mixture from which the steamed bone meal and ground limestone had been omitted. The simple concentrate mixture was made up of two parts ground yellow corn, two parts ground oats, two parts wheat bran and one part linseed meal. This mixture was modified slightly from time to time, as the availability of certain ingredients was limited. To both the basic calf starter and the simple concentrate mixture, the experimental phosphates were added as follows:

Group 1 (steers 49 and 50) 2 per cent of fused tricalcium phosphate containing 0.24 per cent of fluorine.

Group 2 (steers 51 and 52) 1 per cent of mixed phosphate containing 2.0 per cent of fluorine.

Group 3 (steers 53 and 47) 2 per cent of mixed phosphate containing 2.0 per cent of fluorine.

The mixed phosphate was prepared by mixing raw rock phosphate containing 3.51 per cent of fluorine and fused tricalcium phosphate containing 0.02 per cent of fluorine. Thus, the three grain mixtures contained 0.0048, 0.02 and 0.04 per cent of fluorine, respectively. The amount of grain fed was increased periodically to meet the increased maintenance and growth requirements. At the age of 3.5 years the steers were receiving 8 lb. of grain daily. Periodic examinations of the incisor teeth, the legs and the ribs of the steers were made and the results recorded. Weight and height-at-withers measurements were taken monthly at the beginning of the experiment, with the frequency decreasing slightly toward the end of the period.

The steers were slaughtered in October, 1947, when they were between 42 and 44 months of age. The liver, heart, spleen, kidneys and suprarenal glands were examined macroscopically at the time of slaughter. The left foreleg and the upper and lower jaws were cleaned of all flesh and outer connective tissue, the metacarpal being cut longitudinally, and the parts photographed. Before cutting, the smallest circumference of the diaphysis and the circumference in the region of the epiphyseal-diaphyseal junction were determined. A sample of bone was taken from a cross section of the left radius, and the lower left first molar and the lower left third molar were prepared for analysis. The bone samples were analyzed for fluorine, calcium and phosphorus, and the tooth samples for fluorine only. Blood plasma calcium and inorganic phosphorus were determined on samples of blood collected at the end of the experiment.

The growth data were analyzed by analysis of covariance, using simple linear regression for the weight-age relationship. In the case of height at withers, a curvilinear relationship was apparent, and height was plotted against the logarithm of age. The compositions of the bone and tooth samples were treated by analysis of variance with individual comparisons between groups made by separating the individual degrees of freedom for treatment.

RESULTS

Growth. The regression coefficients for weight on age for each individual steer, the average regression coefficient for each group of two, and the average coefficient for all steers are presented in table 1, together with the regression of height on the logarithm of age. The correlation coefficients for both height and weight, also presented in table 1, indicate quite clearly the close fit of these regression curves to the actual data. The analysis of covariance of height-at-withers indicated highly significant differences between the regression coefficients of the individual steers but no significant difference among the three group regressions. In regard to weight, neither individual nor group regressions were significantly different.

TABLE 1

Correlation and regression coefficients for weight-age and height-log age relationships for individual steers and for groups

	Weight-age		Height-log age	
	Regression	Correlation	Regression	Correlation
Steer 49 . . .	26.79	0.993	19.54	0.992
Steer 50	27.63	0.995	23.11	0.995
Av.—group 1	27.21	0.994	21.33	0.989
Steer 51 . . .	28.56	0.993	22.04	0.991
Steer 52	25.25	0.988	20.30	0.995
Av.—group 2	26.90	0.989	21.17	0.991
Steer 53	24.65	0.997	20.89	0.991
Steer 47	27.73	0.986	22.88	0.996
Av.—group 3	26.19	0.981	21.88	0.991
Av.—all animals	26.77	0.988	21.46	0.992

In both the height and weight data, the last three measurements were estimated for steer 50 by the missing plot technique (13), and the degrees of freedom for this steer and for error thus were reduced by 3.

Composition of bones and teeth. The calcium, phosphorus and fluorine content of the samples of bone taken from the left radius, and the fluorine content of the lower left first molar and the lower left third molar are presented in table 2. Statistical analysis indicated highly significant differences among the three groups in the calcium content of the bone samples. The group receiving only 1 per cent of the phosphate in the grain mixture had the highest calcium content in the bone, and this difference is chiefly responsible for the statistically significant difference. The differences in phosphorus content of these bone samples among the groups were not significant.

The fluorine content of the bone samples showed a considerable accumulation of fluorine in the bones, as represented by this one sample, when large amounts of fluorine were fed in the ration. The difference between groups 1 and 2 was statistically significant; the difference between groups 1 and 3 was highly significant; and the difference between groups 2 and 3 was not significant.

TABLE 2

Average composition of bone sample from left radius and of the lower left first and third molar teeth

	Bone—left radius			First molar fluorine	Third molar fluorine
	Calcium	Phosphorus	Fluorine		
	(%)	(%)	(%)	(%)	(%)
Steer 49 . . .	27.0	12.0	0.15	0.17	0.15
Steer 50	26.7	12.8	0.14	0.20	0.19
Av.—group 1	26.8	12.4	0.14	0.18	0.17
Steer 51 . . .	27.5	12.3	0.36	0.39	0.38
Steer 52	27.5	12.6	0.42	0.35	0.34
Av.—group 2	27.5	12.4	0.39	0.37	0.36
Steer 53	26.7	12.1	0.38	0.27	0.25
Steer 47	26.5	12.0	0.61	0.37	0.40
Av.—group 3	26.6	12.0	0.50	0.32	0.32

There was a rather high correlation between the fluorine content of the first and third molars of the same animal. The difference between groups 1 and 2 was significant for both the first and third molar. The difference between groups 1 and 3 was significant for the first molar but not significant for the third molar. The difference between groups 2 and 3 was not significant for either the first or the third molar. The mean values for both first and third molars were slightly lower for group 3 (receiving the most fluorine in the ration) than for group 2 (receiving only half as much).

Examination of bones and teeth. Only one of the bones showed any abnormal characteristics; this was the metacarpal from steer 47 of group 3. The measurements of all of the bones indicated no significant differences, but this one bone (no. 47) was somewhat larger in circumference, both at the smallest

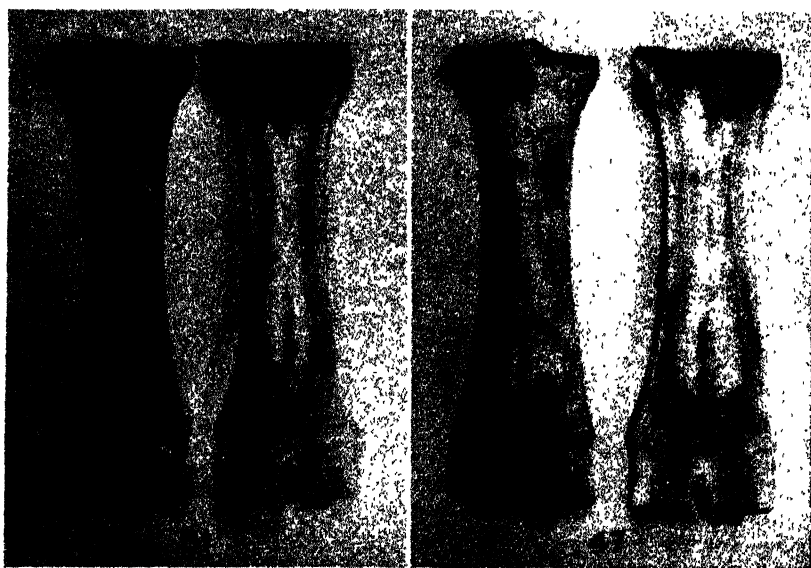


FIG. 1. Enlargement of metacarpal bone due to high fluorine intake of steer 47 compared with the apparently normal bone of steer 51 which was on the borderline level of fluorine intake.

diameter of the diaphysis and the circumference in the region of the epiphyseal-diaphyseal junction. In cleaning this bone, when the periosteum was being removed, a layer of rather porous bone came off with the periosteum in one place. Below this porous layer the bone seemed to be similar in hardness to the bones of the other steers. Careful macroscopic examination showed no differences other than this which could be attributed to the fluorine in the rations. Photographs of the metacarpal bones of steers 51 and 47 are shown in figure 1, to illustrate the difference between an apparently unaffected structure and the enlarged, more porous bone. In the periodic examinations of the steers, no abnormalities of any of the leg bones or ribs, such as exostoses, were observed which could be attributed to the fluorine-containing rations.



FIG. 2. Comparison of roughness, discoloration and softening of incisor teeth on a low fluorine intake (49 and 50), borderline fluorine intake (51 and 52) and high fluorine intake (53 and 47).

The periodic examinations of the incisor teeth indicated that the deciduous teeth of both steers in group 1 showed no abnormalities in the way of roughness or discoloration. The permanent incisors, however, did show some roughness and streaks, especially near the gum line. This is shown in figure 2. Group 2 showed some increased wear and irregularities of the cutting edges of the de-

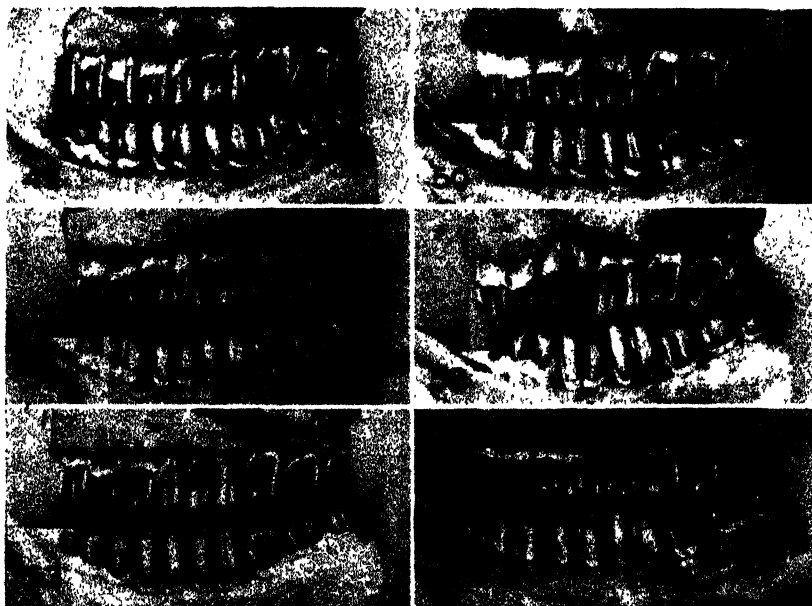


FIG. 3. Comparison of the abrasion of molar and premolar teeth on a low fluorine intake (49 and 50), borderline fluorine intake (51 and 52) and high fluorine intake (53 and 47).

ciduous incisors, and some mottling near the gum. Steer 52 showed somewhat greater mottling than steer 51 and showed wear on the anterior surface of two of the teeth. In group 3 there was definite pitting of the deciduous teeth of both steers and definite roughness and mottling of the permanent teeth. The wear on the anterior surface of the teeth of steer 47 was more pronounced than in steer 52 and was principally on the first pair of teeth.

Photographs of the molar and premolar teeth of the six steers are shown in figure 3. There seemed to be no abnormal wear in either steer of group 1. The abrasion of the teeth of groups 2 and 3 did not differ greatly. The most pronounced wear was found in steer 52 of group 2. Also in this steer the second upper molar on both sides had an abscess at the base, with erosion of the bone surrounding the root. The whole tooth also was discolored, being darker in color than the other teeth.

Examination of internal organs. Steer no. 50 died from ingested wire before the expiration of the experiment, but an autopsy failed to show any noticeable effects of fluorine. Examination of the liver, spleen, kidneys and suprarenal glands of the other five steers failed to show any abnormalities except that the organs were smaller than normally would be found in steers of this size and age. This could be due to the limited amount of exercise allowed. Cysts approximately 0.4 in. in diameter were found in the right atrio-ventricular valves of steers 51 of group 2 and 47 of group 3.

Blood serum. Analysis of the blood serum for calcium and inorganic phosphorus gave values within the normal ranges.

DISCUSSION

Fluorine at the levels studied in this experiment did not affect significantly the rate of growth of steers. At the same age, the heifers in the experiment of Phillips *et al.* (11) showed a definite decrease in weight as compared with the control groups, but these animals had been under the added strain of gestation and lactation. A longer period of development for the steers might have disclosed similar differences.

The statistically significant difference between groups in the calcium content of the bones is difficult to interpret satisfactorily, especially since the group with the highest bone calcium was the one receiving the least calcium supplement in the ration. However, the difference seems to have little practical significance in this experiment. The fluorine content of the bone samples was not strictly proportional to the fluorine intake, but did increase with the increased intake. The fluorine content of the molars did not show a similar proportional relationship, though groups 2 and 3 were higher than group 1.

The incisor teeth of steers 49 and 50 in group 1 did not seem to show any abnormal wear, even though there was a slight roughening and discoloration on the anterior surface. Since these animals had been receiving fluorine almost from birth and through the formative stages of these teeth, which is usually considered the most critical time for such abnormalities to develop, with only

these slight indications of fluorosis, this level of fluorine intake might be considered as a safe level.

Mitchell (10) has emphasized the fact that with the possible exception of very young animals, the phosphorus requirements nearly always are met when a ration contains enough suitable protein supplement to meet the protein requirements of the animal. However, to be on the safe side, many recommendations include some phosphorus supplement in the ration. Such recommendations are seldom for more than 1 per cent of a supplement containing phosphorus at levels comparable to bone meal. In this experiment the low-fluorine ration contained 2 per cent of the fused tricalcium phosphate containing 0.24 per cent fluorine. Apparently, this fused phosphate can be considered as a safe phosphorus supplement, if fed as only 1 per cent of the grain ration.

Since the teeth, both incisors and molars, of the group receiving 0.02 per cent of fluorine in the grain mixture showed definite signs of fluorosis, and the wear of the molars was as severe as for the higher level of fluorine intake, this level should be considered unsafe for dairy animals, especially since milking cows generally would receive larger amounts of grain and consequently a larger total intake of fluorine.

SUMMARY

Six dairy steers received three levels of fluorine in their grain rations (0.0048, 0.02 and 0.04 per cent) from shortly after birth to 3.5 years of age from fused tricalcium phosphate and raw rock phosphate.

The rate of growth of the three groups, as measured by weight and height-at-withers, did not differ significantly.

The fluorine content of the radial bone and the first and third lower molar teeth indicated a definite increase in the deposition of fluorine in these tissues on the fluorine rations, but the increased deposition was not proportional to intake.

Roughness, discoloration and abrasion of the teeth were increased markedly in the two groups receiving the higher levels of fluorine.

It was concluded that fused tricalcium phosphate with 0.24 per cent fluorine is a safe phosphorus supplement if used with discretion.

A grain mixture containing 0.02 per cent or more of fluorine was found to be unsafe for feeding to dairy cattle.

REFERENCES

- (1) BARRENTINE, B. F., MAYNARD, L. A., AND LOOSLI, J. K. The Availability of the Calcium and Phosphorus of Defluorinated Rock Phosphate for the Rat. *J. Nutrition*, 27: 35-42. 1944.
- (2) BIRD, H. R., MATTINGLY, J. P., TITUS, H. W., HAMMOND, J. C., KELLOG, W. L., WEAKLEY, C. E., JR., AND VAN LANDINGHAM, A. H. Nutritive Evaluation of Defluorinated Phosphates and Other Phosphorus Supplements. II. Defluorinated Phosphates as Phosphorus Supplements for Chicks. *J. Assoc. Offic. Agr. Chemists*, 28: 118-129. 1945.
- (3) ELLIS, N. R., CABELL, C. A., ELMSLIE, W. P., FEAPS, G. S., PHILLIPS, P. H., AND WILLIAMS, D. E. Nutritive Evaluation of Defluorinated Phosphates and Other Phosphorus Sup-

- plements. III. Utilization Experiments with Rats. J. Assoc. Offic. Agr. Chemists, 28: 129-142. 1945.
- (4) FRASER, H. F., HOPPE, T. C., SULLIVAN, J. H., AND SMITH, E. R. Phosphate Fertilizers as Possible Source of Phosphorus for Animals. Ind. Eng. Chem., 35: 1087-1090. 1943.
 - (5) GILLIS, M. B., NORRIS, L. C., AND HEUSER, G. F. The Utilization by the Chick of Phosphorus from Different Sources. J. Nutrition, 35: 195-207. 1943.
 - (6) GULLICKSON, T. W., AND OLSON, F. C. Defluorinated Rock Phosphate as a Phosphorus Supplement to the Rations of Dairy Cattle. J. Dairy Sci., 29: 317-320. 1946.
 - (7) HIGNETT, T. P., AND HUBBUCH, T. N. Fused Tricalcium Phosphate. Production by Defluorination of Rock Phosphate in a Shaft Furnace. Ind. Eng. Chem., 38: 1208-1216. 1946.
 - (8) McCONNELL, E. S., INSKO, W. M., JR., AND BUCKNER, G. D. Fused Rock Phosphate for Chicks. Kentucky Agr. Expt. Sta. Bull. 455. 1944.
 - (9) MITCHELL, H. H. The Fluorine Problem in Livestock Feeding. Natl. Research Council; Reprint and Circ. Ser., No. 113. 1942.
 - (10) MITCHELL, H. H. The Mineral Requirements of Farm Animals. J. Animal Sci., 6: 365-377. 1947.
 - (11) PHILLIPS, P. H., HART, E. B., AND BOHSTEDT, G. Chronic Toxicosis in Dairy Cows Due to the Ingestion of Fluorine. Wis. Agr. Expt. Sta. Research Bull. 123. 1934.
 - (12) REED, O. E., AND HUFFMAN, C. F. The Results of a Five-Year Mineral Feeding Investigation with Dairy Cattle. Mich. Agr. Expt. Sta. Tech. Bull. 105. 1930.
 - (13) SNEDECOR, G. W. Statistical Methods, 4th Ed. Iowa State College Press, Ames. 1946.
 - (14) WILLIAMS, D. E., MACLEOD, F. L., MORRELL, E., AND JONES, F. P. Fused Tricalcium Phosphate Fertilizer. Possible Animal Feed Supplement. Ind. Eng. Chem., 38: 651-654. 1946.

THE NATURE OF REPRODUCTIVE FAILURES IN COWS OF LOW FERTILITY¹

T. Y. TANABE AND L. E. CASIDA²

University of Wisconsin, Madison

A realization of the importance of the problem of infertility in dairy cattle has been accentuated by the rapid growth of artificial breeding. The necessary emphasis upon breeding records has made dairymen more aware of the cows which are bred repeatedly and fail to conceive. That approximately 50 per cent of all artificial inseminations are infertile signifies the magnitude of this economic problem.

This study was designed to determine the incidence of fertilization and of early embryonic mortality in "repeat-breeding" cows showing no detectable genital abnormalities. While an attempt was made to investigate some factors associated with infertility in these cows, the major emphasis was given to a consideration of the nature of the interruptions of the reproductive processes.

METHODS AND PROCEDURES

The study was made during the period from November 15, 1947, to April 9, 1948, at the Badger Breeders Cooperative research farm located near Shawano, Wisconsin. A total of 104 repeat-breeding cows, 55 Holsteins and 49 Guernseys, was assembled from 14 of the 23 northeastern Wisconsin counties served by Badger Breeders Cooperative.

The cows were selected by the staff veterinarians of the Cooperative on the following basis: (a) a minimum of four infertile services which would exclude approximately 95 per cent of the cows bred (1), (b) a minimum of one calving which would exclude congenital abnormalities preventing conception, (c) a maximum age limit of 10 years which would exclude infertility resulting from senility, (d) a maximum limit of two cows from any one herd which would minimize the effect of any particular herd management and environment, (e) rejection of cows with gross genital abnormalities detectable by rectal palpation which would tend to exclude cows in which fertilization would be mechanically impossible, (f) exclusion of cows displaying purulent discharges which would screen out obvious conditions for which treatment is indicated, (g) normal lengths of estrual cycles which would eliminate apparent endocrine dysfunction, and (h) normal intervals between breedings which would tend to ex-

Received for publication Sept. 20, 1948.

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Paper No. 383, from the Department of Genetics.

² The authors are deeply indebted to the Badger Breeders Cooperative, Shawano, Wisconsin, and its officers and veterinarians for making the experimental facilities and assistance available and for locating and furnishing the experimental animals. Thanks are due especially to Dr. W. H. Dreher for his willing assistance and cooperation throughout the experiment.

clude infectious diseases such as trichomoniasis. In essence, only those repeat-breeding cows were used which appeared "normal" in all respects at barn examination.

After the cows arrived at the research farm they were checked twice daily for estrual behavior; standing for mounting by other cows was the criterion used to determine estrus. Due to the shortness of the days, heat checks were made at approximately 8:00 a.m. and 4:00 p.m., or at intervals of 8 and 16 hours, respectively. With the observation of the onset of estrus at a particular check, each animal was inseminated at the time of the next check for heat. To standardize the insemination technique one inseminator made the major portion of the inseminations. The semen was diluted with synthetic pabulum (2) at the ratio of 1:30, and the insemination volume kept constant at 1.0 ml. diluted semen. The deposition of semen was confined to the second and third rings of the cervix. Other than meeting the requirements of using "day-of-collection" semen, a random sample of the semen available for field use on that day was used.

The cows then were scheduled to be slaughtered alternately on the third or thirty-fourth day after the experimental breeding, except for deviations necessitated by slaughter facilities. By the third day after breeding fertilization or cleavage of the egg is detectable, yet the ova are in the oviducts which facilitates their recovery. Further, fertilized ova recovered at a later stage could not be distinguished from fragmenting unfertilized ova with much surety. By the 34th day the embryo is of sufficient size to permit gross measurement and macroscopic observation for normality. For the cows which were permitted to go for 34 days before slaughter, routine palpations were made on the 21st, 24th, 27th, 30th, and 33rd day after breeding. The object of this series of palpations was to detect any quiet ovulations which might occur about the time of an expected estrual period.

The cows which, though scheduled for slaughter on the 34th day, returned to estrus were rebred at the time of the next heat check and slaughtered on the third day following. From cows slaughtered on the third day after either the first or second experimental breeding, the reproductive tract was removed, the oviducts severed from the mesosalpinx and flushed with physiological saline solution to recover the ova. When ova were recovered, fertilization was determined by the presence of two or more blastomeres of equal size, as microscopically observed at 440 magnifications. In the cows slaughtered on the 34th day after experimental breeding, the uterine horns were dissected to determine the status of the embryo, if present.

RESULTS

One group of cows was intended to contribute information on the percentage of breedings resulting in normal embryos on the 34th day. A total of 53 animals originally was allotted to this group (table 1), but in the end, one was eliminated and, as a result, the embryo studies included a maximum of 52 cows. The one cow eliminated was found at slaughter to have bilateral tubal obstructions which in itself would have prevented fertilization and embryo formation.

Of the 53 cows, only 26 actually were slaughtered on the 34th day, since the remainder returned to estrus between the 11th and 32nd day after the first experimental breeding. Normal embryos were present in only 12 of the 26 cows on the 34th day; the crown-rump measurements on these embryos ranged from 12–17 mm.³ Abnormal embryos were present in eight other cows, four with embryos normal in size but showing a more or less generalized hemorrhagic condition and the other four with embryos small in size (7–11 mm.) and obviously disintegrating. Six cows slaughtered at 34 days had no embryos; two of these had pyometra. Of the other four, one had a quiet ovulation between the time of breeding and time of slaughter. No such occurrence was detected in the other cows by palpations of the ovaries on the 21st, 24th, 27th, 30th, and 33rd days after experimental breedings.

A group of 27 cows returned in estrus 11 to 32 days after breeding (table 1),

TABLE 1

Cows intended to furnish information on normality of embryo on the 34th day

Pregnancy status of cow	Guernsey		Holstein	
	No.	%	No.	%
Normal embryo	5	20.0	7	25.0
Hemorrhagic embryo	2	8.0	2	7.1
Disintegrating embryo	2	8.0	2	7.1
No embryo	2	8.0	2	7.1
Pyometra	1	4.0	1	3.6
Estrual recurrence (before 34th day)	13	52.0	14*	50.0
Total	25	100.0	28	99.9

* Includes one cow which was eliminated from the study of embryo normality at the 34th day, because when slaughtered 3 days after the second experimental breeding she was found to have bilateral tubal obstructions.

indicating that the breedings on them resulted in no embryos by 34 days. These animals then were experimentally rebred with the intention of their yielding information on the rate of fertilization when later slaughtered on the third day.

A second group of 51 cows (table 2) was slaughtered on the third day after the first experimental breeding to yield information on the rate of fertilization. Obvious causes for the failure of fertilization formed the basis for eliminating five of these animals: one failed to ovulate, one had bilateral tubal obstructions, and three had unilateral tubal obstructions on the ovulating side. Six other cows were eliminated because of a lack of information on fertilization. In three of these animals no ova were found, although the animals were free of genital abnormalities, and in the other three only empty zona pellucidas, two of which contained embedded spermatozoa, were found. Elimination of these latter six animals, in effect, charges the lack of fertility information on them to imperfec-

³ Acknowledgement is gratefully made to Dr. S. H. McNutt, of the Department of Veterinary Science, for his assistance in determining the normality of embryos.

tions of the recovery technique rather than to some factor which also affects fertilization rate. There remained, then, 40 animals slaughtered on the third day after the first experimental breeding that actually furnished fertilization information.

Another group of cows yielding fertilization information was mentioned above in connection with the animals intended to furnish information on the percentage of embryos at 34 days. These animals were the 27 cows which returned to estrus before the allotted 34 days and were experimentally bred for the second time (table 2). Actually only 19 of these animals furnished information on fertilization rate. Of the remaining eight animals, no ova were recovered from four cows free of genital abnormalities on the ovulating side.

TABLE 2
Cows intended to furnish information on fertilization rate

Fertilization data	Slaughtered 3 days after:							
	1st exptl. breeding				2nd exptl. breeding			
	Guernsey		Holstein		Guernsey		Holstein	
	No.	%	No.	%	No.	%	No.	%
Contributing information:								
Fertilized or unfertilized ova								
No genital abnormality	20	83.3	19	70.4	11	84.6	8	57.2
Unilateral tubal abnormality (non-ovulating side)	0	0.0	1	3.7	0	0.0	0	0.0
Contributing no information:								
Empty zona pellucida	1	4.2	2	7.4	0	0.0	0	0.0
No ova recovered:								
No genital abnormality	2	8.3	1	3.7	2	15.4	1	7.1
Unilateral tubal abnormality (non-ovulating side)	0	0.0	0	0.0	0	0.0	1	7.1
Unilateral tubal abnormality (ovulating side)	1	4.2	2	7.4	0	0.0	1	7.1
Bilateral tubal abnormality	0	0.0	1	3.7	0	0.0	1	7.1
Ovulation failure	0	0.0	1	3.7	0	0.0	2	14.3
Totals	24	100.0	27	100.0	13	100.0	14	99.9

Ovum recovery was not possible in the other four cows due to a unilateral tubal obstruction on the ovulating side in one, bilateral tubal obstructions in another, and ovulation failure in two.

Altogether then, the pregnancy and fertilization studies utilize data on a maximum of 52 animals on the 34th day and 59 animals on the third day after experimental breeding. Of the 59 animals, the first experimental breeding yielded data on 40 cows. The other 19 had previously contributed information on the normality of embryos at 34 days and now contributed information on the rate of fertilization on the third day after the second experimental breeding.

In subsequent analyses summary totals which fall short of these two maximum totals, 52 and 59, or a grand total of 111, for the 3-day and 34-day post-breeding groups, respectively, are due to a lack of collateral information for the particular criterion under consideration.

TABLE 3
Genital Abnormalities

Genital Abnormalities	Guernsey		Holstein	
	No.	%	No.	%
Absent	48	98.0	45	81.8
Present				
Ovulation failure			3	5.5
Tubal obstructions				
Unilateral	1	2.0	5	9.1
Bilateral			2	3.6
Totals	49	100.0	55	100.0

Genital abnormalities. An attempt was made to exclude cows having gross abnormalities from the sample of animals selected for this study. Tubal obstructions not readily palpable appeared, however, in eight of the 104 cows slaughtered (table 3). Six of these were unilateral, and two were bilateral, and each of sufficient severity to act as a definite barrier to the transport of the ovum or sperm through the oviduct. These cases fell in the general categories of salpingitis, hydrosalpinx and bursitis. In addition to these presumed permanent tubal abnormalities, there were three cases of ovulation failure which may be considered as temporary barriers to fertilization.

Altogether, out of a total of 104 cows, 10.6 per cent were found with genital abnormalities. Their distribution between breeds was strikingly unequal. Genital abnormalities occurred in 18.2 per cent of the Holsteins, whereas such occurrences were found in only 2.0 per cent of the Guernseys.

Breeds. Slaughter data on 31 Guernseys and 28 Holsteins (table 4) have shown, first, that there are no statistically significant differences between the fertilization rates of ova recovered on the third day after the first and the second experimental breedings (60.0 vs. 63.6 for the Guernsey and 70.0 vs. 75.0 for the Holstein). On this basis, all cows slaughtered on the third day after experimental breeding, whether first or second, have been pooled.

With the division by breeds, the combined rates of fertilization for first and second experimental breedings were 61.3 for the Guernsey and 71.4 for the Holstein. The percentages of normal embryos at 34 days were 20.0 and 25.9

TABLE 4
Breeds

Breed	3 days after:				34 days after:	
	1st exptl. breeding		2nd exptl. breeding		1st exptl. breeding	
	Total no. cows	% with fertilized ova	Total no. cows	% with fertilized ova	Total no. cows	% with normal embryos
Guernsey	20	60.0	11	63.6	25	20.0
Holstein	20	70.0	8	75.0	27	25.9
Totals	40	65.0	19	68.4	52	23.1

for the respective breeds. Statistical significance of the differences between breeds could not be established on the present numbers.

In view of the insignificant differences between the breeds, subsequent analyses will be made by pooling all cows of both breeds furnishing information at the same stage.

• *Bang's disease.* For the 110 cows tested for Bang's disease the following percentages were observed: 71.8 negative, 20.9 positive, and 7.3 suspect. A higher incidence of Bang's disease (positive and suspect reactors) occurred in the Holstein breed (37.0) than in the Guernsey breed (19.6). No explanation for this difference is apparent. All cases of anatomical genital barriers were found in cows which were negative to Bang's disease.

Agglutination test information for Bang's disease was available on 58 cows of the 3-day group and on 52 cows of the 34-day group. No difference in the fertilization rates was found between the 41 cows of the negative group (65.9) and the 17 cows of the positive-suspect group (64.7). Among the cows slaughtered on the 34th day after the experimental breeding the percentage of the 38 cows in the negative group having normal embryos was 26.3, and of the 14 in the positive-suspect group, 14.3. This difference is not statistically significant.

Number of previous services. While the minimum number of infertile services specified for the experimental animals was four, the average number per cow prior to the experimental breeding was 6.4. The Guernseys averaged 6.8 with a range of 4 to 11, while the Holsteins had an average of 6.0 with a range of 4 to 13.

The 59 cows of the 3-day group and the 52 cows of the 34-day group were pooled and arrayed on the basis of the number of services which each had received previously. Division of these 111 cows into low and high groups, as nearly equal in size as possible without splitting the median class, fell between six and seven previous services. No significant difference in the fertilization rates was found between the 34 cows with four to six previous services and the 25 cows with seven to thirteen previous services (67.6 vs. 68.0 on the third day). The percentage of normal embryos on the 34th day in the 34 cows of the low group was 23.5 and in the 18 cows of the high group, 22.2.

Number of previous calvings. The 107 cows for which reproductive histories were available averaged 2.8 previous calvings with an insignificant difference between breeds, being 2.9 for the Guernsey and 2.7 for the Holstein. These cows were arrayed according to their respective number of calvings and divided into a low group (1 and 2 calvings) and a high group (3 to 6 calvings). The 28 cows with a low number of calvings and slaughtered on the third day showed a higher fertilization percentage than did the 28 cows with a high number of calvings (75.0 vs. 57.1), but this difference is not statistically significant ($P = 0.2-0.1$). Similarly, in the group of cows slaughtered on the 34th day, a higher percentage having normal embryos was found in the 25 cows with a low number of calvings (32.0) than in the 26 cows with a high number of calvings (15.4). This difference of 16.6, again, is not statistically significant ($P = 0.3-0.2$).

Herd size. The average size of herd from which the experimental animals

originated was 20.6 cows. The Guernsey herds ranged from 3 to 63 cows with an average of 20.5, as compared with the Holstein herds which ranged from 7 to 70 cows with an average of 20.8. Again all the cows were pooled and arrayed according to the size of herd from which they came. There were 56 cows from small herds (3-19 cows) and 55 cows from large herds (20-70 cows). The fertilization percentage in the 33 cows from small herds was 60.6 and in the 26 cows from the large herds, 73.1. This difference of 12.5 per cent is not statistically significant ($P = 0.5-0.3$). In the 23 and the 29 cows slaughtered on the 34th day from small and large herds, respectively, the percentages with normal embryos were 26.1 and 20.7, with a difference of 5.4 which also is insignificant.

Herd breeding index. To obtain a more accurate differentiation between "problem cows" from good breeding herds and representative cows from "problem herds," herd indexes were calculated for the calendar year 1947. These indexes were simply the percentages of cows receiving "first services" for which no repeat breedings were required during a minimum period of 3 months. The average herd index was 43.3 per cent. Based on their respective herd breeding indexes, all cows were arrayed and divided into a low- and a high-index group. The low-index group consisted of 56 cows from herds with breeding efficiencies ranging from 0.0 to 45.5, while the 55 cows comprising the high-index group came from herds with breeding efficiencies ranging from 46.2 to 75.0. Twenty-nine cows from low index herds had a fertilization rate of 44.8 and thirty cows from high index herds, 86.7. This difference of 41.9 is highly significant statistically ($P = 0.01$). On the other hand, there is no significant difference between the percentages of cows having normal embryos at 34 days from the low index herds (22.2 on 27 cows) and from the high index herds (24.0 on 25 cows).

DISCUSSION

Cows with genital abnormalities detectable by palpation intentionally were excluded from this study so that chief emphasis could be given to a determination of the relative importance of failure of fertilization and early embryonic death. The finding of 7.7 per cent of the animals with tubal abnormalities was not expected. Inasmuch as these abnormalities probably are permanent and not amenable to treatment, the problem of their diagnosis is particularly important so that such cows can be removed from the herd. Rowson (3) has outlined procedures for detecting these conditions, which, if applied skillfully, should eliminate many such animals. The frequency observed in this study undoubtedly represents only the less readily detectable abnormalities. It will be assumed, however, that this frequency can serve as an index of the relative frequencies of all grades of the abnormalities in groups of animals under comparison.

The higher incidence of genital abnormalities in the Holstein breed (18.2 per cent) than in the Guernsey breed (2.0 per cent) is surprising in view of the generally-recognized lower breeding efficiency of the Guernsey than of the Holstein. The fact that no tubal abnormalities were found in cows reacting to

the agglutination test for Bang's disease also is at variance with the idea that brucellosis increases the incidence of salpingitis.

The comparison of different groups can be seen better if estimates of embryonic degeneration and mortality are available alongside the data on fertilization rate. These estimates (table 5) are derived from the differences observed in the original data between the fertilization rate and the percentage of cows with normal embryos at 34 days. This difference, for example, with cows negative to the test for Bang's disease (see above) was 65.9 minus 26.3 or 39.6. From this figure then an estimate of the percentage of embryonic death and abnormalities at the 34th day, $60.1 \left(\frac{39.6}{65.9} \times 100 \right)$, is calculated (table 5).

In practically all artificial breeding associations the Guernsey breed consistently has maintained a lower breeding efficiency than the Holstein breed.

TABLE 5
Group Comparisons

Comparison	Fertilization rate (%)	% embryonic death and abnormality at 34 days
Breed		
(Guernsey vs. Holstein)	61.3 vs. 71.4	67.4 vs. 63.7
Bang's Disease		
(Negative vs. Positive-Suspect)	65.9 vs. 64.7	60.1 vs. 77.9
Number previous services (4-6 vs. 7-13)	67.6 vs. 68.0	65.2 vs. 67.4
Number previous calvings (1-2 vs. 3-6)	75.0 vs. 57.1	57.3 vs. 73.0
Herd size		
(3-19 vs. 20-70)	60.6 vs. 73.1	56.9 vs. 71.7
Herd breeding index (0.0-45.5% vs. 46.2-75.0%)	44.8 vs. 86.7	50.4 vs. 72.3

The differences observed in this study between the breeds fail to explain this field condition. The higher rate of fertilization and the lower rate of embryonic mortality in the Holstein (table 5) are offset by the higher percentage of genital abnormalities noted above.

The view commonly is held that infection of a herd by Bang's disease causes a reduction in the conception rate. In this study, little difference was found between the fertilization rates of the negative group (65.9) and the positive-suspect group (64.7). However, a suggestion of a higher rate of embryonic mortality in the positive-suspect group (77.9) than in the cows of the negative group (60.1) was observed.

Barrett *et al.* (1) found a progressively declining rate of pregnancy (as diagnosed by manual palpation at 34 to 50 days after breeding) with each additional service. Such a trend was not noted in this group of experimental cows. In fact, no appreciable differences (table 5) between the fertilization rates or between the rates of embryonic mortality were found between the cows with a low number of previous services (4-6) and those with a high number of services (7-13). The exclusion of cows with genital abnormalities from the present

population but not from that studied by Barrett and associates may account for the difference in the performance of the two populations.

The most obvious effects upon both fertilization rate and embryonic mortality appear to be produced by the three factors: age of cow (number of previous calvings), herd size and herd breeding index. The younger animals had a higher fertilization rate and at the same time a lower embryonic death rate than the older animals (table 5). Cows from the larger herds and from herds with higher breeding indexes had the higher fertilization rates. Concurrently, however, the same cows had the higher embryonic death rates, which by the 34th day left them with no more normal embryos than cows from small herds and from herds of low breeding indexes.

The small number of animals used in this study makes it difficult to study the interactions of the various factors upon fertilization rate and embryonic death rate. The most interesting finding resulting from attempts at further study has been the correlation between age of animal and size of herd from

TABLE 6

The nature of reproductive failures in cows of low fertility

		%
Failure of fertilization		39.7
Physical barriers absent	31.0	
Physical barriers present	8.7	
Bilateral	1.9	
Unilateral (ovulating side)	3.9	
Ovulation failure	2.9	
Embryonic abnormalities or mortality before 34 days		39.2
Embryos still normal at 34 days		21.1
Total		100.0

which it came. The larger herds furnished 64.2 per cent of the younger animals and the smaller herds furnished 58.9 per cent of the older animals. Other factors showed less association among themselves. There is then some confounding of youngness of animal and largeness of herd. Additional data will be necessary for further pursuance of this analysis.

Cows of low fertility may be divided into three main categories on the basis of their reproductive performance during the first 34 days after breeding: (a) failure of fertilization, (b) embryonic abnormalities and mortality before 34 days, and (c) embryos still normal at 34 days (table 6).

Throughout the major portion of this study the cows showing definite physical barriers to fertilization have been excluded. It can be seen from table 3 that out of 104 cows there were three cases of ovulation failure, two of bilateral tubal obstructions and six of unilateral tubal obstructions, but of the last only four (table 2) were on the ovulating side. A failure of fertilization in 8.7 per cent of the cows would be expected because of physical barriers: 1.9 per cent because of bilateral tubal obstructions, 3.9 per cent because of unilateral tubal obstructions on the ovulating side and 2.9 per cent because of ovulation failure.

Subtraction of the percentage of physical genital barriers (8.7) from all animals would leave 91.3 per cent of the animals mechanically capable of fertilization. Since the pooled fertilization percentage for genitally-normal animals of both breeds (3 days after the first and second experimental breedings) is 66.1 (table 4), its complementary percentage for non-fertilization in genitally-normal animals is 33.9. In terms of all animals as a whole, this becomes 33.9×91.3 , or 31.0 per cent which fail to conceive although genital barriers are absent (table 6), or a combined percentage of 39.7 ($31.0 + 8.7$) with and without physical obstructions in which there is fertilization failure.

The percentage of cows having normal embryos at 34 days was 23.1 (table 4). Again in terms of the whole population of cows, 91.3×23.1 or 21.1 is the calculated percentage with normal embryos at 34 days.

From the percentage of fertilization failure (39.7) and the percentage of cows with normal embryos at 34 days (21.1) the rate of embryonic mortality may be derived by subtracting the sum of these two percentages from 100. The remainder, 39.2, is an indirect estimate of embryonic abnormalities and mortality by the 34th day after breeding.

SUMMARY

The study covered 104 cows, 49 Guernsey and 55 Holstein, each of which had been bred from 4 to 13 times without conceiving. The percentage of genitally-normal cows having fertilized ova when slaughtered at 3 days was 66.1, but at 34 days the percentage having normal embryos had dropped to 23.1, for an embryonic death rate of 65.1. Estimates were made of the effect of the following factors on fertilization and embryonic death: (a) breed, (b) Bang's disease, (c) number of previous services, (d) number of previous calvings, (e) herd size, and (f) herd breeding index. Appreciable effects upon the fertilization rate were noted for the first and the last three factors, and upon embryonic death rate for the second and the last three. Visible genital abnormalities were found in 10.6 per cent of the cows at the time of slaughter; these abnormalities were not detected by clinical examination. There was a higher occurrence in the Holsteins (18.2 per cent) than in the Guernsey (2.0 per cent). The total percentage of cows with genital abnormalities, 10.6, included 8.7 per cent in which the abnormality constituted a physical barrier to fertilization. Considering the group of cows as a whole, including those with genital abnormalities, division may be made into three main categories on the basis of reproductive performance during the first 34 days after breeding: (a) failure of fertilization, 39.7 per cent; (b) embryonic abnormalities and mortality before 34 days, 39.2 per cent; and (c) embryos still normal at 34 days, 21.1 per cent.

REFERENCES

- (1) BARRETT, G. R., LLOYD, C. A., AND CARPENTER, R. A. Order Number of Insemination and Conception Rate. *J. Dairy Sci.*, 31: 683. 1948.
- (2) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (3) ROWSON, L. E. A. Sterility in Cattle due to Bursitis and Salpingitis. *Vet. Record*, 54: 74-75. 1942.

GROWTH AND PRODUCTION OF INBRED AND OUTBRED HOLSTEIN-FRIESIAN CATTLE¹

W. J. TYLER,² A. B. CHAPMAN, AND G. E. DICKERSON³

University of Wisconsin, Madison

One of the greatest limitations to genetic improvement of our livestock for economically important characters is the relative inaccuracy in evaluating the individual animal's transmitting ability. The most complete information on the genetics of the sire or dam, if they are not inbred, supplies limited evidence of the breeding worth of an individual offspring. The individual's own appearance or performance may supply more information on its transmitting ability, but this also usually is far from an accurate guide.

On theoretical grounds, selection alone generally should improve the average desirability but have little effect on the uniformity of the offspring. Inbreeding alone has been found to cause individuals to breed more nearly true for good and poor inheritance alike. This means that there is less genetic variation in estimates of transmitting ability. Hence, selection combined with inbreeding should be a means of producing animals which transmit desirable traits more uniformly than would selected or unselected outbred individuals.

The rate of inbreeding which can be practiced without danger of deterioration depends on the degree of dominance (including overdominance), the real merit of the foundation stock and the heritabilities and intensities of selection for the desired traits. The possibilities and limitations of selection and inbreeding as a method for the improvement of dairy cattle could be determined by a carefully planned selection and inbreeding program with the best foundation stocks available. Until this is done systematically, some evidence can be secured from herds in which inbreeding has been practiced and in which there are enough non-inbred control data to warrant conclusions.

Data of this kind are presented in this paper for Holstein-Friesian cattle in three unrelated herds. Comparisons are made between non-inbred cattle and inbreds of different degrees of inbreeding on body dimensions at 6 months of age, 18 months of age and at maturity. Similar data also are presented on milk and butterfat production and butterfat test.

REVIEW OF LITERATURE

An inbreeding experiment with Jersey cattle, started by Regan at New Jersey and then moved to California, had as its objective the development of bulls prepotent for high production. It was reported in 1944 (1) that out of 50 bulls that had progeny tests, only four failed to raise the production of their daughters

Received for publication September 23, 1948.

¹ Paper No. 376 from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin.

² Assistant Professor, Dairy Husbandry Department, West Virginia University.

³ Associate Professor, Animal Husbandry Department, University of Missouri.

over dams. The average increase in butterfat production of daughters over dams was 88 lb. During the course of the inbreeding experiment, several heritable defects have been reported (7, 8, 10, 11, 12, 15, 16).

Baker *et al.* (2) studied the growth curves of inbred and outbred daughters of one Holstein-Friesian bull and found significant decreases in height at withers, weight and heart girth with increases in the coefficient of inbreeding.

Bartlett *et al.* (3, 4, 5, 9) attempted to establish a herd of Holstein-Friesian cattle possessing genetic factors for high milk production and high fat test. Four sires and 45 high-producing cows were selected as foundation animals, but only one of the four sires withstood the inbreeding tests. The descendants of this one sire were inbred up to 20 per cent (Wright's (21) coefficient). They did not differ significantly in body weight or body dimensions at any stage from birth to maturity. Females inbred more than 20 per cent were smaller at maturity than the outbreds used as controls. The average milk and butterfat production of the outbred daughters of four sires was significantly larger than that of the inbreds, but the average fat tests for the two groups were about the same.

Starting with cows of average production, Woodward and Graves (20) used inbreeding to determine whether a good dairy herd could be developed from an ordinary one by the use of only one foundation sire. Grade cows were bred either to a purebred Guernsey or purebred Holstein-Friesian bull, and the daughters bred back to their sires for successive generations. They found that intense inbreeding (25 to 50) resulted in lighter birth weights and a reduction in mature weights. A few inbred Guernseys were deformed at birth and the average mortality of the calves after birth was greater among the inbreds. The effects on production of inbreeding and breed differences were confused in these data.

Plum (13) found a negative intra-sire correlation between inbreeding and butterfat production in one Jersey herd in which the inbreeding ranged from 0 to 22 per cent.

Tyler *et al.* (19) found an average intra-sire decline of 0.28 lb. in birth weight for each increase of 1 per cent inbreeding in data from three unrelated herds.

EXPERIMENTAL PROCEDURE

The data were collected from 1937-1945 on the Holstein-Friesian cattle of the three herds described in a previous paper (19).

Body dimensions were taken at approximately 6 and 18 months of age, and 1 month following each calving. Only females were measured at ages beyond 6 months. The dimensions taken were height at withers, circumference of shin bone, heart girth and width at hips. The measurements that were taken after each calving were adjusted to a 60-months-of-age basis.

The monthly milk weights and fat tests were taken directly from the D.H.I.A. herd books, and the summation of the first ten testing-day values multiplied by 30.5 was used to estimate 305 days' production (18). All production records were standardized to a mature equivalent, twice-a-day milking, 305-day basis.

The suggested breeding plan in each herd was to mate the herd sires to their

TABLE 1

Means of the body dimensions of inbred and outbred males at 6 months of age

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(cm)	(cm)	(cm)	(cm)
Inbreds					
6 to 11	10	104	14.2	124	29.9
12 to 17	15	104	14.0	123	29.7
18 to 23	7	105	14.2	122	30.1
24 to 29	10	101	13.8	119	29.4
30 to 37	1	105	14.5	125	32.5
All inbreds	43	103	14.1	122	29.8
Outbreds	17	104	13.9	123	30.1

daughters or close collateral relatives as a test of their actual breeding worth, and during the same period mate them to unrelated cows to produce offspring or control animals by the same sire. The inbreeding percentages were calculated by Wright's method, and during the period of investigation these inbreeding percentages varied from 0 to 37. For comparative purposes animals whose inbreeding percentages were less than six were classified as outbreds. Individuals whose inbreeding percentages were six or larger were designated as inbreds.

RESULTS

Dimensions at 6 months of age. There were 60 males and 193 female calves that were measured at approximately 6 months of age. The number of calves and the means of the four body dimensions for the outbred and inbred groups are given for males in table 1 and for females in table 2. The inbred animals were divided into five groups on the basis of the size of their inbreeding percentages. The figures in tables 1 and 2 suggest that, with the exception of the circumference of shin bone measurement for male calves, the average inbred was slightly smaller than the average outbred individual.

In order to remove any bias that sire, dam and herd may have had on these

TABLE 2

Means of body dimensions of inbred and outbred females at 6 months of age

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(cm)	(cm)	(cm)	(cm)
Inbreds					
6 to 11	39	100	13.1	119	29.4
12 to 17	43	102	13.2	120	30.1
18 to 23	18	101	13.0	119	29.5
24 to 29	12	100	13.0	119	30.0
30 to 37	5	101	13.3	118	29.5
All inbreds	117	101	13.1	119	29.7
Outbreds	76	102	13.4	122	30.5

TABLE 3

Intra-sire correlations and partial regression coefficients (holding mature body dimension of dam constant) between body dimensions of male and female calves at 6 months of age and inbreeding

Dimension	Males			Females		
	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding
Height at withers	55	-0.138	-0.055	180	0.084	0.045
Circum. of shin bone	55	-0.078	-0.002	180	-0.016	-0.001
Heart girth	55	-0.205	-0.148	180	-0.003	-0.002
Width at hips	55	-0.093	-0.017	180	-0.019	0.005

average values, the influence of inbreeding on each body dimension was measured by the within-sire partial regression of dimension on inbreeding, holding the same mature dimension of the dam constant. The correlation and partial regression coefficients for each dimension are given separately for each sex in table 3. The partial regression coefficients for the males were negative, but small and not statistically significant. In the case of the heifer calves, the small negative and positive within-sire partial regression coefficients were not significant. Hence, inbreeding was not demonstrated to have influenced the size of male and heifer calves at 6 months of age by these data.

Dimensions at 18 months of age. A total of 152 females were measured at 18 months of age. Sixty-four of these heifers were outbred. The inbreeding percentages on the other 88 animals ranged from 6 to 34 with an average of 14. The number of heifers and the means of the four body dimensions at 18 months of age are given for the outbred and inbred heifers in table 4. The average dimensions of all inbreds were the same as those for the outbreds. The within-sire partial regression coefficients of each dimension on inbreeding, holding constant the same dimension of the dam at maturity, were calculated and are given

TABLE 4

Means of the body dimensions of inbred and outbred females at 18 months of age

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(cm)	(cm)	(cm)	(cm)
Inbreds					
6 to 11	27	125	17.0	169	44.6
12 to 17	36	125	16.9	169	45.1
18 to 23	17	125	16.7	169	44.9
24 to 29	5	122	17.1	163	44.0
30 to 34	3	126	17.0	165	45.3
All inbreds	88	125	16.9	168	44.9
Outbreds	64	125	16.9	168	44.9

TABLE 5

Intra-sire correlations and partial regression coefficients (holding mature body dimension of dam constant) between body dimensions of heifers at 18 months of age and inbreeding

Dimension	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding
Height at withers	141	0.048	0.007
Circumference of shin bone	141	0.021	0.002
Heart girth	141	-0.167 ^a	-0.106 ^a
Width at hips	141	-0.012	-0.002

^a $P < .05$.

in table 5. The partial regression of heart girth on inbreeding was significant and negative, but the other regressions were not significant. The reduction in heart girth amounted to about 1 cm. or about 20 lb. in body weight for an increase of 10 per cent in inbreeding.

Dimensions at maturity. The average of the four dimensions for the 56 inbred and 55 outbred cows that were measured after one or more calvings are given in table 6. The inbreds were smaller on the average than the outbreds in circumference of shin bone, heart girth and width at hip measurements. The correlations and partial regressions of dimensions on inbreeding, holding dam's mature dimension constant, are shown in table 7. The within-sire regression coefficients are negative, but not statistically significant.

Production records. Milk and butterfat production records were available on 47 outbred and 42 inbred daughters of 5 sires. The average milk and butterfat production and butterfat test for outbred and inbred daughters by each sire and the partial regression of production and test on inbreeding, holding corresponding performance of the dam constant, are given in tables 8, 9 and 10.

The outbred daughters produced more milk than their inbred half-sisters in four out of five sire groups. The partial regressions were significant for one of

TABLE 6

Means of the body dimensions of inbred and outbred females at maturity

	No. of animals	Dimension			
		Height at withers	Circumference shin bone	Heart girth	Width at hips
		(cm)	(cm)	(cm)	(cm)
Inbreds					
6 to 11	16	136	17.5	190	57.2
12 to 17	19	137	17.1	189	56.5
18 to 23	13	139	17.3	186	56.1
24 to 29	7	134	17.8	191	56.8
30 to 34	1	138	18.7	201	61.6
All inbreds	56	137	17.4	189	56.8
Outbreds	55	136	17.9	193	57.8

TABLE 7

Intra-sire correlations and partial regression coefficients (holding mature body dimension of dam constant) between body dimensions at maturity and inbreeding

Dimension	Degrees of freedom	Correlation between dimension and inbreeding	Partial regression of dimension on inbreeding
Height at withers	104	0.013	-0.003
Circumference of shin bone	104	-0.067	-0.011
Heart girth	104	-0.085	-0.082
Width at hips	103	-0.175	-0.026

TABLE 8

Number and mean milk production of outbred and inbred daughters and their dams by sires, and the partial regressions (holding milk production of the dams constant) of milk production on inbreeding (mature equivalent, twice-a-day, 305-day records)

Sire	Outbred			Inbred				Partial regression BF on inbreeding
	No. daughters	Daughter av.	Dam av.	No. daughters	Av. % inbreeding	Daughter av.	Dam av.	
		(lb.)	(lb.)			(lb.)	(lb.)	
1	4	15,394	13,818	13	16	12,685	13,486	- 149.2 ^a
2	5	12,368	12,637	15	9	12,132	13,116	- 93.0
3	4	12,739	11,606	4	11	11,128	11,444	- 154.6
4	31	11,447	12,066	6	24	10,306	10,188	- 51.0
5	3	10,988	10,882	4	12	11,068	11,969	12.9
Total	47				14			- 73.8 ^b

^a $P > 0.01 < 0.05$.

^b $P < 0.01$.

TABLE 9

Number and mean butterfat production of outbred and inbred daughters and their dams by sires, and the partial regression (holding dams' butterfat production constant) of butterfat production (BF) on inbreeding (mature equivalent, twice-a-day, 305-day records)

Sire	Outbred			Inbred				Partial regression BF on inbreeding
	No. daughters	Daughter av.	Dam av.	No. daughters	Av. % inbreeding	Daughter av.	Dam av.	
		(lb.)	(lb.)			(lb.)	(lb.)	
1	4	518	485	13	16	446	471	-6.81 ^a
2	5	496	484	15	9	512	459	-0.66
3	4	432	383	4	11	372	378	-6.22
4	31	381	405	6	24	338	363	-1.82
5	3	386	371	4	12	382	377	-0.30
Total	47			42	14			-2.32 ^b

^a $P > 0.01 < 0.05$.

^b $P < 0.01$.

the sires, while the average decline (within-sire) for all sires was 74 lb. of milk for each 1 per cent increase in inbreeding. The average regression coefficient was highly significant.

The average butterfat production of the outbreds was greater than that of the inbreds for four of the five sires, but the partial regressions were significant for only one sire (table 9). The average regression within-sire was significant and amounted to 2.3 lb. decrease for every 1 per cent increase in inbreeding. The average butterfat production for the inbred daughters of sire 2 was 16 lb. more than the outbreds. This superiority was caused by the high fat production of the slightly inbred (6 per cent) daughters. However, the more highly inbred cows were lower producers which led to the negative regression for this group of 20 daughters.

TABLE 10

Number and average fat percentage of outbred and inbred daughters and their dams by sires and the partial regressions (holding dams' fat percentage constant) of fat percentage on inbreeding

Sire	Outbred			Inbred			Partial regres- sion fat test on inbreeding	
	No. daughters	Daughter av.	Dam av.	No. daughters	Av. % inbreed- ing	Daughter av.		Dam av.
		(%)	(%)			(%)	(%)	
1	4	3.36	3.51	13	16	3.52	3.49	0.006
2	5	4.01	3.83	15	9	4.22	3.50	0.014
3	4	3.39	3.30	4	11	3.34	3.30	- 0.008
4	31	3.33	3.36	6	24	3.28	3.56	- 0.010 ^a
5	3	3.51	3.41	4	12	3.45	3.15	0.012
Total	47			42	14			0.0053

* $P > 0.01 < 0.05$.

The mean fat test of the outbreds was higher in 3 out of the 5 daughter groups (table 10). The increase, however, was significant for only one sire. The average regression of fat test on inbreeding was positive but not statistically significant.

DISCUSSION

The body size of the inbred Holstein-Friesian cattle in the three herds studied was slightly less than the size of outbred animals in the same herds at ages of 6 months, 18 months and maturity when the records were adjusted to a standard mature size of their dams.

The influence of inbreeding on birth weights of animals in these three herds had been found previously to be significant and amounted to a decrease of 2.3 lb. for a 10 per cent increase in the coefficient of inbreeding (19). The lack of evidence of an inbreeding effect at 6 months of age may have been due to the small numbers, the low degree of inbreeding in most comparisons, the differential culling between inbreds and outbreds or, of course, an absence of an inbreeding depression in some of these progeny groups. Selection does not appear to have been a factor, however. For example, there was no evidence that the difference between the

birth weights of inbred culls and inbred non-culls was any larger than the difference between the birth weights of outbred culls and outbred non-culls. However, the percentages of culling for inbred and outbred groups of males were 61 and 40, respectively. For the female groups the percentages were 31 and 24. This may mean that differential culling between inbreds and outbreds, as far as dimensions at older ages are concerned, has occurred even though it is not apparent from the birth weights of culls and non-culls.

The inbred and outbred cattle, in general, were similar in body size at the various ages to the "normal" size of Holstein-Friesian animals as given by Ragsdale's (14) tables for height at withers, circumference of chest (heart girth in this study) and width at hips. The exceptions to the normal were: (a) Both inbred and outbred male calves and inbred female calves at 6 months of age were about 4 per cent smaller in heart girth. (b) The inbred and outbred heifers at 18 months of age were approximately 2 per cent larger in heart girth. (c) At maturity the inbred cows average 2 per cent narrower at the hips and 3 per cent smaller in heart girth measurement than the normal animal.

The results gave no evidence of an effect of inbreeding on butterfat test but did indicate that the milk and butterfat production of the inbred cows was significantly lower than the production of the outbred cows by the same sires. On the basis of these results, the average rate of decline of butterfat production in these cows was sufficiently low so that with moderate inbreeding (6 per cent per generation) the decrease in production should be 14 lb. of butterfat per generation. The standard deviation of butterfat in these cows is about 80 lb., and if heritability is 0.2, the parents would need to average $14 \div (80 \times 0.2) = 0.87$ of a standard deviation above the group average to counteract this inbreeding depression. Production can be measured only in the female, and culling among cows based on their own records has limited possibilities (17). This means that bulls and heifers saved for breeding would need to come from dams averaging $14 \div (80 \times 0.1) = 2 \times 0.87 = 1.74$ standard deviations above the average or from the best fifth to tenth of the cows. It would be impossible to do this even if selection of bulls and heifers were based entirely on production records, which it cannot be. A more realistic intensity of inbreeding that could be offset by selection would be about 3 per cent per generation. However, there was considerable variation between sires in the effect of inbreeding on production, presumably because of the level of transmitting ability of the sire relative to the average of the unrelated dams to which he was mated. Thus, by linebreeding to the superior sires, linebred families of increased uniformity of transmitting ability probably could be produced without much loss of production.

SUMMARY

Growth and production data of inbred and outbred progeny of Holstein-Friesian sires in three Wisconsin State Department of Public Welfare herds were collected and analyzed to determine the effect of inbreeding on body dimensions (height at withers, circumference of shin bone, heart girth and width of hips) at 6 and 18 months of age and at maturity and on milk and butterfat production

and butterfat test. The average intra-sire partial regression (holding mature size of dam constant) of dimension on inbreeding was used to measure this effect. The partial regression of dimensions on inbreeding was essentially zero, except for heart girth at 18 months and maturity, which was significant only at 18 months. Intra-sire partial regressions (holding dam's record constant) of milk and butterfat production of 42 inbred and 47 outbred cows on inbreeding were significant and amounted to an average decrease of 74 lb. of milk and 2.3 lb. of butterfat for each 1 per cent increase in inbreeding. No evidence of an effect of inbreeding on butterfat percentage was indicated.

Considerable variation was found in the partial regression coefficients of sires, indicating that offspring of some sires could be inbred without any apparent decrease in body size or production, possibly in part because the beneficial effect of increasing the relationship to a sire transmitting a superior level of production tends to offset detrimental effects of inbreeding to him.

ACKNOWLEDGMENTS

The authors are indebted to Mr. W. W. Kinyon, Farm Supervisor, Wisconsin State Department of Public Welfare, for making available the records on which this study is based. They wish to thank the herdsmen at Winnebago State Hospital, Southern Wisconsin Colony, and the Oregon State Farm for their aid in the collection of the data.

REFERENCES

- (1) ALBAUGH, R. Victory-bred Cattle. *Hoard's Dairyman*, 89: 294. 1944.
- (2) BAKER, G. A., MEAD, S. W., AND REGAN, W. M. Effect of Inbreeding on the Growth Curves of Height at Withers, Weight and Heart Girth of Holstein Females. *J. Dairy Sci.*, 28: 607-610. 1945.
- (3) BARTLETT, J. W., AND MARGOLIN, S. A Comparison of Inbreeding and Outbreeding in Holstein-Friesian Cattle. *N. J. Agr. Expt. Sta. Bull.* 712. 1944.
- (4) BARTLETT, J. W., REECE, R. P., AND LEPARD, O. L. The Influence of Inbreeding on Birth Weight, Rate of Growth and Type of Dairy Cattle. *J. Animal Sci.*, 1: 206-212. 1942.
- (5) BARTLETT, J. W., REECE, R. P., AND MIXNER, J. P. Inbreeding and Outbreeding Holstein-Friesian Cattle in an Attempt to Establish Genetic Factors for High Milk Production and High Fat Test. *N. J. Agr. Expt. Sta. Bull.* 667. 1939.
- (6) DICKERSON, G. E. Effects of Inbreeding in Dairy Cattle. *J. Dairy Sci.*, 23: 546. 1940.
- (7) GREGORY, P. W., MEAD, S. W., AND REGAN, W. M. A New Type of Recessive Achondroplasia in Cattle. *J. Heredity*, 33: 317-322. 1942.
- (8) GREGORY, P. W., MEAD, S. W., AND REGAN, W. M. A Congenital Hereditary Eye Defect of Cattle. *J. Heredity*, 34: 124-128. 1943.
- (9) MARGOLIN, S., AND BARTLETT, J. W. The Influence of Inbreeding Upon the Weight and Size of Dairy Cattle. *J. Animal Sci.*, 4: 3-12. 1945.
- (10) MEAD, S. W., GREGORY, P. W., AND REGAN, W. M. Proportionate Dwarfism in Jersey Cows. *J. Heredity*, 33: 411-416. 1942.
- (11) MEAD, S. W., GREGORY, P. W., AND REGAN, W. M. Hereditary Congenital Flexed Pasterns in Jersey Cattle. *J. Heredity*, 34: 367-372. 1943.
- (12) MEAD, S. W., GREGORY, P. W., AND REGAN, W. M. A Recurrent Mutation of Dominant Achondroplasia in Cattle. *J. Heredity*, 37: 183-188. 1946.
- (13) PLUM, MOGENS. Production in a Large Jersey Herd as Affected by Sires, Dams and Yearly Variations. *Proc. Am. Soc. Animal Production* (1933), 26: 53-57. 1934.

- (14) RAGSDALE, A. C. Growth Standards for Dairy Cows. Mo. Agr. Expt. Sta. Bull. 336. 1934.
- (15) REGAN, W. M., GREGORY, P. W., AND MEAD, S. W. Hereditary Strabismus in Jersey Cattle. J. Heredity, 35: 233-234. 1944.
- (16) REGAN, W. M., MEAD, S. W., AND GREGORY, P. W. An Inherited Skin Defect in Cattle. J. Heredity, 26: 357-362. 1935.
- (17) SEATH, D. M. The Intensity and Kind of Selection Actually Practiced in Dairy Herds. J. Dairy Sci., 23: 931-951. 1940.
- (18) TYLER, W. J., AND CHAPMAN, A. B. A Simplified Method of Estimating 305-day Lactation Production. J. DAIRY SCI., 27: 463-469. 1944.
- (19) TYLER, W. J., CHAPMAN, A. B., AND DICKERSON, G. E. Sources of Variation in the Birth Weights of Holstein-Friesian Calves. J. DAIRY SCI., 30: 483-498. 1947.
- (20) WOODWARD, T. E., AND GRAVES, R. R. Some Results of Inbreeding Grade Guernsey and Grade Holstein-Friesian Cattle. U. S. Dept. Agr. Tech. Bull. 339. 1933.
- (21) WRIGHT, SEWELL. Coefficient of Inbreeding and Relationship. Am. Naturalist, 56: 330-338. 1922.

A STUDY OF FEEDING LOW LEVELS OF THYROPROTEIN TO DAIRY COWS FOR A PERIOD OF FIFTY-TWO WEEKS^{1, 2}

R. G. SWANSON AND C. B. KNOTT

Pennsylvania Agricultural Experiment Station, State College

Considerable research has been conducted in efforts to determine the value of synthetic thyroproteins in the rations of dairy cows (1, 2, 3, 4, 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29). In the majority of these reports, it appears that with the levels of thyroprotein fed there were losses in body weight, increases in heart and respiration rates, increases in body temperature and irregular responses in milk and milk fat production.

As a result of the previous review of available literature, it appeared to be desirable to conduct experiments for a minimum of 1 year using somewhat lower levels of thyroprotein feeding than those reported previously. The effects upon milk and milk fat production, as well as upon body weights, body temperatures, heart rates, respiration rates and breeding efficiency, were observed and are presented in this report.

EXPERIMENTAL PROCEDURE

Two trials were conducted to determine the effects of feeding levels of 0.625, 1.25 and 5.0 g. of iodinated casein daily to cows for a period of 52 weeks. Two separate experiments were conducted under somewhat different feeding conditions and the results are presented in this report.

Trial I. Four groups of three Holstein cows each were selected so as to be comparable in age, stage of lactation, stage of gestation, body weight and milk production. The four groups were assigned at random to the three levels of thyroprotein as well as to a control group. All cows in this trial were maintained at a T.D.N. intake of 125 per cent of Morrison's (10) recommended standards for good cows under usual conditions. The rates of feeding were recalculated every 2 weeks on the basis of body weight and milk and milk fat production during the previous 3 days. The cows were milked twice daily and their production recorded for each milking. The percentage of milk fat was determined once each month by the Babcock method. Body weights were checked every 2 weeks and rectal temperatures every 3 months. A stethoscope was used to check the heart rates every 3 months. Management conditions were controlled as carefully as possible so as to be similar for all cows on the trial.

Trial II. Four groups of eight cows, each including the Ayrshire, Brown

Received for publication Oct. 8, 1948.

¹ Taken from data presented in a thesis to the graduate faculty of The Pennsylvania State College by R. G. Swanson in partial fulfillment of the requirements for the Degree of Master of Science. Authorized for publication on August 23, 1948 as paper No. 1464 in the journal series of the Pennsylvania Agricultural Experiment Station.

² The authors wish to express their appreciation to the Whitmoyer Laboratories, Inc., Myerstown, Pa. for a research grant and to the Cerophyl Laboratories, Inc. which supplied the iodinated casein for this study.

Swiss and Guernsey breeds, were selected and assigned at random to the same levels of thyroprotein with a control group as in trial I. All cows in this trial were maintained at a T.D.N. intake of 110 per cent of Morrison's (10) recommended standards for good cows under usual conditions. As in trial I, the rates of feeding were recalculated every 2 weeks on the basis of body weight and milk and milk fat production during the previous 3 days. The cows were milked three times per day, and milk weights were recorded at each milking. Fat tests were determined at 10-day intervals during the trials and for 8 days before and after the withdrawal of thyroprotein from the ration. Body weights were checked every 2 weeks and rectal temperatures were taken every month. Heart and respiration rates also were checked once a month with a stethoscope. Management conditions were controlled carefully so as not to be a variable in the experiment. All milk records in both trials were converted to a 4 per cent fat-corrected milk, mature equivalent basis (5, 8). All data were analyzed according to the methods of Snedecor (23) wherever applicable.

EXPERIMENTAL RESULTS

Trial I. When milk production of the groups receiving thyroprotein was compared to the control group, no statistically significant differences were observed. There was considerable variation between cows within groups. In table 1 are presented the average total milk production and the average number of days

TABLE 1
Mean total milk production^a trial I

Thyroprotein fed	Milk	Milk fat	Days in milk
(g.)	(lb.)	(%)	
0 (control)	14,708	3.76	328
0.625	14,311	3.71	305
1.25	13,844	4.04	309
5.0	14,456	4.09	300

^a Expressed as 4 per cent F.C.M., M.E.

milked during the feeding trial.

The analysis of variance of the data relative to the milk production of the cows in trial I is presented in table 2. While there apparently were differences

TABLE 2
Analysis of variance of milk production of cows in trial I

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	143	68,522,262	
Cows	2	727,489	363,745
Months	11	18,616,342	1,692,395 ^a
Treatments	3	771,770	257,257
C × M	22	8,291,638	376,893
C × T	6	10,027,368	1,671,228 ^a
T × M	33	6,209,044	188,152
Remainder	66	23,878,611	361,797

^a = Significant at 1 per cent level.

TABLE 3
Mean body weights in trial I

Thyroprotein fed	Initial	Final	Mean gain
(g./day)	(lb.)	(lb.)	(lb.)
0 (control)	1,418	1,608	190
0.625	1,334	1,509	175
1.25	1,247	1,388	141
5.0	1,395	1,522	127

in the fat content of the milk produced by the various groups, when the production was converted to 4 per cent fat-corrected milk these differences were not significant.

All cows in this trial gained in body weight during the experiment. The average group weights and gains are presented in table 3. The differences in the average group gains are not statistically significant, but a trend appears, since the group receiving the most thyroprotein gained the least and the control group gained the most. The analysis of variance of the data relative to the effects upon body weight in trial I are presented in table 4.

TABLE 4
Analysis of variance of body weights in trial I

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	323	5,802,954	
Periods	26	770,528	29,636 ^a
Cows	2	30,982	15,491
Treatments	3	1,248,055	416,018
Treatments × Cows	6	2,026,303	337,717 ^a
Treatments × Periods	78	188,560	2,417
Cows × Periods	52	396,785	7,630
Sampling error	156	1,141,741	7,319

^a = Significant at 1 per cent level.

While there was a highly significant difference in the body weights of the cows during the different periods, the differences between cows and between treatments were not statistically significant. The interaction of treatments and cows was highly significant.

Body temperatures, heart rates and respiration rates of the cows in this trial, as presented in table 5, showed no significant increases or changes when compared

TABLE 5
The effect of thyroprotein feeding upon body temperature, heart rate, and respiration rate in trial I

Thyroprotein fed	Body Temperature	Heart rate	Respiration rate
(g./day)	(° F.)	(min.)	(min.)
0 (control)	101.6	65.8	23.3
0.625	101.7	65.7	24.1
1.25	101.8	65.3	24.9
5.0	101.6	65.1	23.8

TABLE 6
Mean total milk production^a in trial II

Thyroprotein fed	Milk	Milk fat	Days dry
(g./day)	(lb.)	(%)	
0 (control)	9,876	4.3	77
0.625	12,564	4.4	46
1.25	11,461	4.3	61
5.0	10,586	4.4	59

^a Expressed as 4 per cent F.C.M., M.E.

with the control cows. There was no indication that the amounts of thyroprotein fed had any adverse effect on breeding efficiency.

Trial II. Although there was some differences in the average total milk production (table 6) between the groups receiving thyroprotein and the control group, an analysis of variance (table 7) showed these differences to be non-

TABLE 7
Analysis of variance of milk production of cows in trial II

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	383	91,616,562	
Cows	7	1,522,412	217,487
Treatments	3	2,444,977	814,992
Months	11	16,886,612	1,535,146 ^a
C × T	21	6,922,541	329,644 ^b
C × M	77	18,960,403	246,239 ^b
T × M	33	2,719,377	82,405
Sampling error	231	42,160,240	182,511

^a = Significant at 1 per cent level.

^b = Significant at 5 per cent level.

significant statistically. Some of the variation between groups can be accounted for by the differences in the length of dry periods (table 6).

On the basis of the mean per cent of milk fat, there was no effect of feeding these levels of thyroprotein upon the fat content of the milk produced. There were no significant differences between cows relative to milk production in trial II.

In table 8 are presented data relative to the milk production of five cows of the

TABLE 8
Average milk production of comparable groups of 5 cows from each group during first 4 months of thyroprotein feeding in trial II^a

Thyroprotein fed	Av. production 10 d. prior to start	Av. total production (4 mo.)	Av. production 10 d. prior to end
(g./day)	(lb.)	(lb.)	(lb.)
0 (control)	44.6	4,682	34.1
0.625	45.5	5,540	44.6
1.25	43.8	5,084	38.6
5.0	43.4	5,241	43.2

^a = Expressed as 4 per cent F.C.M.

TABLE 9

Analysis of variance of data of 4 months milk production at beginning of trial

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	239	2,320,113	
Days	11	27,298	2,482 ^a
Cows	4	1,216,734	304,183 ^a
Treatments	3	128,429	42,809
T × C	12	691,029	57,586 ^b
T × D	33	35,329	1,071
C × D	44	49,469	1,124
Sampling error	132	171,825	1,302

^a = Significant at 5 per cent level.^b = Significant at 1 per cent level.

various groups during the first 4 months of trial II. These cows were selected solely on the basis of obtaining groups which were comparable in milk production, stage of lactation, stage of gestation and age. On the basis of the mean data it would appear that some stimulation of milk production occurred as a result of thyroprotein feeding. However, an analysis of variance (table 9) of the data proved the differences to be non-significant statistically.

Near the conclusion of the 52-week feeding period, cows in the same stage of

TABLE 10

Comparison of production during the 35 days preceding and following withdrawal of thyroprotein from the ration

Thyroprotein fed	Mean decline in production per cow ^a
(g./day)	(lb.)
0 (control)	31
0.625	66
1.25	42
5.0	152

^a Expressed as lb. 4 per cent F.C.M., M.E. during the 35-day period.

lactation were selected from each group and compared for 35 days before and after the withdrawal of thyroprotein from the ration. The results of this study (table 10) indicate that there may have been some stimulation to milk production as a result of thyroprotein feeding. When thyroprotein was withdrawn from the ration there was no decline in the per cent of milk fat. This seems to indicate that, at these levels of intake, thyroprotein did not increase the percentage of milk fat.

TABLE 11

Mean body weights in trial II

Thyroprotein fed	Initial	Final	Mean gain
(g./day)	(lb.)	(lb.)	(lb.)
0 (control)	1,140	1,285	145
0.625	1,130	1,230	100
1.25	1,180	1,240	60
5.0	1,155	1,235	80

TABLE 12
Analysis of variance of body weights in trial II

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	831	30,940,085	
Weeks	25	1,159,127	46,365 ^a
Cows	7	17,828,396	2,546,914
Treatments	3	79,099	26,366
T × C	21	9,845,110	468,815 ^a
T × W	75	109,643	1,461
C × W	175	715,418	4,088 ^a
Sampling error	525	1,203,292	2,292

^a = Significant at 1 per cent level.

All cows in trial II gained in weight during the experiment (table 11). Analysis of variance (table 12) of body weights showed that there were no significant differences between treatments. The interaction of treatments and cows was highly significant, indicating that individual cows responded differently in weight gains to thyroprotein feeding.

In trial II the body temperatures, heart rates and respiration rates were checked once each month. Body temperatures varied considerably from month to month but not between treatments. Heart and respiration rates of the cows receiving thyroprotein were not significantly different from those in the control

TABLE 13
Mean body temperature, heart and respiration rate (trial II)

Thyroprotein fed	Body temperature	Heart rate	Respiration rate
(g./day)	(° F.)	(min.)	(min.)
0 (control)	101.6	74	32
0.625	101.6	76	33
1.25	101.5	76	31
5.0	101.6	76	32

group; however, individual cows showed variations in response as indicated by a highly significant interaction between treatments and cows. The groups as a whole, however, did not exhibit these differences (table 13).

In trial II, as in trial I, no indication of lowered breeding efficiency could be observed due to thyroprotein feeding.

SUMMARY

Thyroprotein in the form of iodinated casein fed at levels of 0.625, 1.25 and 5.0 g. daily to dairy cows with a T.D.N. intake of 125 per cent of Morrison's standards for good cows under usual conditions (10) produced no significant stimulation of milk production, milk fat production, heart rates, respiration rates or body temperatures. Gains in body weight and breeding efficiencies were not affected significantly.

These same levels of thyroprotein feeding accompanied by a T.D.N. intake of 110 per cent of Morrison's standards for good cows under usual conditions (10)

produced increases in milk production which approached statistical significance. Milk production appeared to decline when thyroprotein was withdrawn from the ration. Mean milk fat percentage, body weights, heart rates, respiration rates, body temperatures and breeding efficiencies were not affected significantly by these levels of thyroprotein administration.

REFERENCES

- (1) BLAXTER, K. L. Stimulation of the Milk Production of Dairy Cows by Feeding Thyroid-Active Iodinated Proteins. *Nature*, Lond., 152: 751-752. 1943.
- (2) BLAXTER, K. L. The Effect of Iodinated Protein Feeding on the Lactating Cow. *J. Endocrinol.* 4: 237-266. 1945.
- (3) BLAXTER, K. L. Experiments with Iodinated Casein on Farms in England and Wales. *J. Agr. Sci.*, 36(2): 117-150. 1946.
- (4) BOOTH, A. N., ELVEHJEM, C. A., AND HART, E. B. Some Effects of Feeding Iodinated Casein to Dairy Cows. *J. Dairy Sci.*, 30(7): 443-455. 1947.
- (5) GAINES, W. S. Relation Between Percentage Fat Content and Yield of Milk. Correction of Milk Yield for Fat Content. Ill. Agr. Expt. Sta. Bull. 245. 1923.
- (6) HIBBS, J. W., AND KRAUSS, W. E. The Effects of Thyroprotein on Milk Production and on Some of the Constituents of the Milk and Blood of Dairy Cows. *J. Animal Sci.*, 6(2): 161-173. 1947.
- (7) KEMMERER, A. R., BOLOMEY, R. A., VAVICK, M. G., AND DAVIS, R. N. Effect of Thyroprotein on Vitamin Content of Milk. *Proc. Soc. Exptl. Biol. Med.*, 63: 309-310. 1946.
- (8) KENDRICK, J. F. Standardizing Dairy Herd Improvement Association Records in Proving Sires. Bureau of Dairy Industry, Mimeograph 925.
- (9) MOORE, L. A. Some Physiological Effects of Feeding Thyroprotein to Dairy Cows. *J. Dairy Sci.*, 29(8): 532-533. 1946.
- (10) MORRISON, F. R. Feeds and Feeding, 20th Ed. Morrison Publishing Co., Ithaca, N. Y.
- (11) REECE, R. P. The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows over a Three Week Period. *J. Dairy Sci.*, 27(7): 545-550. 1944.
- (12) REECE, R. P. The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows over Extended Periods. *J. Dairy Sci.*, 29(8): 533. 1946.
- (13) REECE, R. P. The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows over an Extended Period. *J. Dairy Sci.*, 30(5): 313-324. 1947.
- (14) REECE, R. P. The Influence of Thyroprotein in the Ration of Dairy Cattle. *J. Dairy Sci.*, 30(8): 574. 1947.
- (15) REED, O. E. Report of the Chief of the Bureau of Dairy Industry. Agr. Research Admin. Published by U.S.D.A. Sept. 1, 1947.
- (16) REINEKE, E. P. The Effect of Thyroprotein on Milk Production, Metabolism, and Growth. *J. Dairy Sci.*, 25(8): 701. 1942.
- (17) REINEKE, E. P. Practical Trials on the Use of Synthetic Thyroprotein for Increased Production of Milk and Butterfat. *J. Dairy Sci.*, 26(8): 750-751. 1943.
- (18) REINEKE, E. P., HERMAN, H. A., TURNER, C. W., AND RAGSDALE, A. C. Stimulation of Milk and Butterfat Production in Cows Fed Varying Levels of Synthetic Thyroprotein. *J. Animal Sci.*, 3(4): 439-440. 1944.
- (19) REINEKE, E. P., AND TURNER, C. W. Formation in Vitro of Highly Active Thyroproteins, Their Biological Assay, and Practical Use. Mo. Agr. Expt. Sta. Research Bull. 355. 1942.
- (20) REINEKE, E. P., AND TURNER, C. W. Increased Milk and Fat Production Following the Feeding of Artificially Formed Thyroprotein. *J. Dairy Sci.*, 25(5): 393-400. 1942.
- (21) SEATH, D. M., BRANTON, C., AND GROTH, A. H. The Response of Louisiana Milk Cows to Iodinated Casein Feeding. *J. Dairy Sci.*, 27(8): 641-642. 1944.
- (22) SEATH, D. M., BRANTON, C., AND GROTH, A. H. The Response of Louisiana Milk Cows to Iodinated Casein Feeding. *J. Dairy Sci.*, 28(7): 509-517. 1945.

- (23) SNEDECOR, G. W. *Statistical Methods*. Ia. State College Press. 1940.
- (24) SYKES, T. F., WRENN, T. R., MOORE, L. A., AND THOMAS, J. W. The Effect of Thyroprotein and Feed Intake on the Heart Rate of Dairy Steers. *J. Dairy Sci.*, 30(8): 576. 1947.
- (25) THOMAS, J. W., AND MOORE, L. A. Some Effects of Feeding Thyroprotein to Dairy Cows. *J. Dairy Sci.*, 30(8): 575-576. 1947.
- (26) VANLANDINGHAM, A. H., HENDERSON, H. O., AND WEAKLEY, C. E. Effect of Iodinated Casein on Milk and Butterfat Production and on the Ascorbic Acid Content of the Milk. *J. Dairy Sci.*, 27(5): 385-396. 1944.
- (27) VANLANDINGHAM, A. H., HYATT, G., AND WEAKLEY, C. E. Effect of Feeding Iodinated Casein to Dairy Cows on the Protein Composition and Content of Milk. *J. Dairy Sci.*, 29(8): 533. 1946.
- (28) VANLANDINGHAM, A. H., HYATT, G., WEAKLEY, C. E., AND HENDERSON, H. O. Further Observations on the Effects of Feeding Thyroprotein to Dairy Cows. *J. Dairy Sci.*, 30(8): 576-577. 1947.
- (29) VANLANDINGHAM, A. H., HYATT, G., WEAKLEY, C. E., AND HENDERSON, H. O. Effect of Thyroprotein on Milk, Butterfat Production, and Health of Cow and Calf. Paper presented at Northeastern States Colleges Feed Conference Board Meeting, Dec. 8-9, 1947.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

APRIL, 1949

NUMBER 4

FACTORS MODIFYING THE EXCRETION OF FECAL ANDROGENS IN THE COW

R. B. CASADY, H. H. COLE AND G. H. HART

Division of Animal Husbandry, University of California, Davis

The function of androgens in the female is becoming recognized as one of considerable importance. Accumulating evidence indicates that androgens may play an important role in determining the normal processes associated with the estrous cycle. Salmon and Geist (23) have shown that androgens stimulate sexual libido in the human female and Cole *et al.* (2) report similar results with the ewe. Selye (24) states that androgens are a potent means of terminating menstruation and that testosterone produces mammary development in the female rat.

Since McGee (19) first extracted the male hormone from bull testes in 1927, investigations of androgen excretion have been confined almost entirely to the urine. In 1942 Riley and Hammond (22) showed, however, that the feces of dairy cows had considerable androgenic activity as determined by precocious comb and wattle growth in chicks given cow feces in the diet. There was also a retardation of gonadal development of the chicks. Feces of cows both with and without access to pasture contained the active principle in similar concentrations, a fact leading the authors to conclude that androgen is not derived from the diet. On the other hand, bull feces showed no evidence of androgen content. Turner (25 to 29) and Longwell and Gassner (8, 18), have confirmed and extended the findings of Riley and Hammond.

The purpose of the present study is to investigate factors modifying the fecal excretion of androgens in the cow, and to determine the possible relationship between the fecal androgen level and certain reproductive disorders in cattle.

EXPERIMENTAL

The animals used in these studies were Jersey and Holstein cows from the Division of Animal Husbandry herd with the exception of four Jerseys and one Guernsey with cystic ovaries purchased from nearby breeders. The method of assay used was that described by Riley and Hammond (22), with the exception that day-old White Leghorn cockerels were used in place of Rhode Island Red chicks and the feeding period was shortened to 14 days. White Leghorns were used because they were most readily available at the time the study was initiated. Dorfman (4) has shown since that the White Leghorn comb is 10 to

Received for publication August 24, 1948.

20 times more responsive to androgen than the comb of the Rhode Island Red or Barred Rock. The shorter feeding period was instituted when it was noticed that the comb response at the end of 14 days was great enough to afford a reliable assay. Only cockerels were used, any pullets found at autopsy being discarded. Data from cockerels weighing less than 90 g. at autopsy were not included. It was intended to use ten or eleven chicks for each test but the number was reduced in some instances to seven by the limitations imposed by sex and size.

To rule out the possible effect of environment, a control group of chicks receiving no feces was run with each test. There was no evidence to indicate that changes in environment or in chicks used influenced the results.

The feces were collected in most instances in a clean container as they were dropped. In a few instances, part of the sample was obtained by artificial evacuation of the bowel; in a few others, samples were taken up from a clean concrete floor. The feces were dried in shallow pans in ovens at a temperature of 39 to 41° C. for 48 to 72 hours and then ground in a hammer mill before mixing with the chick ration.

The basal chick ration was a well-balanced diet furnished by the Poultry Division of the University of California.¹ The feces were mixed with the basal ration in the proportion of 10 per cent by weight and fed to day-old chicks. Fed in this proportion, the average consumption of feces was 18 g. per bird, with extremes of 15 to 21. There was as much variation in feces consumption among groups of chicks on the same feces sample as among groups on different samples. As differences between the means of only 2 of 18 repeat tests of given samples were significant, it is apparent that variations in feed consumption were not sufficient to be of great importance in the interpretation of the results. At the end of 14 days on feed, the combs were removed as described by Frank *et al.* (6). The testes were removed for weighing at the same time. The comb and testes were weighed separately on a torsion balance to the nearest 0.2 mg.

The chicks were kept in a Jamesway brooder with the temperature under the hover pad at 95 to 100° F. The room temperature was maintained at approximately 70° F.

EXPERIMENTAL RESULTS

Accuracy of the assay method. To test the accuracy of the androgen assay procedure, 18 of the feces samples were tested twice, using two groups of chicks at different times. During both feeding periods, conditions were duplicated as closely as possible with respect to ration, temperature, number of birds and length of feeding period. The mean comb weight of the repeat group was compared with that of the original assay group. Statistical analysis of the difference between the means showed that in 16 out of 18 compared assays the difference between the means was not significant at the 5 per cent level of probability

¹ The basal ration consisted of: fishmeal-16, meat scraps-16, soybean meal-56, alfalfa meal-20, salt-2, limestone and $MnSO_4$ -4, fish oil-1, millrun-60, brewer's yeast-4, ground wheat-40, ground corn-173, and whey-8.

(table 1). Thus, this method of assay for fecal androgens is fairly reliable and significant differences in comb weights usually may be attributed to differences in the amount of androgens in the feces fed. At the present time no adequate explanation is available as to why significant differences were found between mean comb weights in two of the repeat tests.

Effect of diet on the fecal androgen level. Assuming that the 24-hour excretion of androgens is constant, greater androgenic activity per gram should be obtained from feces of cows on a low fiber diet with low total feces during the 24 hours than from cows on a roughage diet with a larger amount of feces

TABLE 1

Tests on the accuracy of fecal androgen assay by the chick comb growth method with feces fed as 10% of the diet^a

Sample no.	Feces consumption per chick for the 2 tests:		Average comb weights per 100 g. body weight for the two tests:		Significance of difference between duplicate tests expressed as probability values ^b
	Original	Repeat	Original	Repeat	
	(g.)	(g.)	(mg.)	(mg.)	
2	18.2	15.3	71.2	71.7	> 0.50
6	19.7	16.7	105.6	100.8	> 0.50
3	16.9	17.6	322.2	290.5	> 0.50
15	18.4	17.7	252.7	276.3	> 0.50
19	18.2	19.4	228.2	214.2	> 0.50
18	17.0	18.4	272.9	268.4	> 0.50
5	17.2	16.7	271.7	246.1	> 0.50
8	16.6	16.4	279.4	246.5	> 0.50
31	18.9	19.7	250.2	245.8	> 0.50
32	18.5	c	429.3	409.7	> 0.50
4	17.9	15.2	333.5	284.4	0.37
12	18.1	17.4	325.2	282.6	0.37
17	18.5	18.1	244.3	212.6	0.36
13	15.9	19.5	110.0	158.7	0.20
33	17.3	c	403.2	321.0	0.20
30	15.3	19.3	159.9	237.2	0.20
28	15.2	20.6	119.9	254.1	0.05
16	19.0	17.8	200.9	125.4	< 0.01

^a Duplicate tests were made on given samples with at least 1 week between tests. An average of 9 chicks were used for each test with extremes of 7 to 11.

^b Probability values greater than 0.05 indicate that the difference between duplicate tests is not significant.

^c Data not available.

voided. To test this hypothesis, two non-lactating cows were placed on a cement floor without bedding and fed alfalfa hay and straw in excess for a preliminary period of 3 days, at the end of which time a 24-hour collection of feces was made and an aliquot taken for assay. All hay and straw then was taken away and each cow was fed 5 lb. of rolled barley twice daily for a preliminary period of 3 days, at the end of which time another 24-hour collection of feces was made. The experiment was repeated using two other cows with a preliminary feeding period of 5 days for alfalfa hay alone and 4 days for grain. Again 24-hour collections of feces were made at the end of each feeding period and aliquots taken for assay. During all feeding periods the cows had access

TABLE 2

Effect of a roughage diet as compared to a grain diet on the fecal androgen level in cows as determined by the chick comb growth method^a

Cow donor	Samples compared	Feces consumption per chick receiving feces of cows on:		Av. comb wt. per 100 g. body wt. with feces from cows receiving:		Significance of difference between means:	
		Rolled barley ^b	Roughage ^c	Rolled barley ^b	Roughage ^c	t	P
		(g.)	(g.)	(mg.)	(mg.)		
562	28 & 30	15.2	15.3	119.9	159.9	1.15	> 0.2
562	28 & 30	20.6	19.3	254.1	237.2	0.22	> 0.5
E-59	27 & 29	17.3	16.4	62.2	117.2	3.93	< 0.01
757	45 & 46	19.2	19.4	187.2	461.0	4.99	< 0.01
821	43 & 44	20.5	18.0	99.7	534.1	6.45	< 0.01

^a The dried feces were fed as 10% of the chick ration for 14 days. An average of 9 chicks were used per test.

^b 5 lb. twice daily.

^c Cows 562 and E-59 received alfalfa hay and straw in excess.

Cows 757 and 821 each received 10 lb. of alfalfa hay twice daily.

to no other food, and water and salt were furnished *ad libitum*. Contrary to the hypothesis mentioned, the feces of the cows on a diet of roughage showed greater androgenic activity than feces of cows on the grain diet in three of four instances (table 2). The differences in comb weight produced by the feces of cow 562 on the two diets are not significant at the 5 per cent level of probability, but those for cows E-59, 757 and 821 are highly significant at the 1 per cent level. No explanation is available at present to account for the difference in comb weight between the original and repeat tests on each diet in the case of cow 562. On the hay diet, the total amounts of feces excreted during the 24-hour period were 2.6, 3.8, 3.8 and 4.3 times greater than on grain alone for cows 562, E-59, 757 and 821, respectively (table 3). Thus, considering the greater androgen content per gram of feces in three out of four cases while on the hay diet, it is apparent that the total fecal androgen excretion is more than three times that on the grain diet. These data clearly indicate that diet has a promi-

TABLE 3

Influence of diet on 24-hour excretion of feces

Cow donor	Dry weight of feces on:		Per cent increase
	Rolled barley ^a	Roughage ^b	
	(g.)	(g.)	
562	1350	3505	260.0
E-59	1145	4320	380.0
757	815	3140	380.0
821	795	3420	430.0

^a 5 lb. twice daily.

^b Cows 562 and E-59 received alfalfa hay and straw in excess.

Cows 757 and 821 each received 10 lb. alfalfa hay twice daily.

nent role in determining the fecal androgen level. This effect of diet must be considered in comparative assays. All comparative assays made during the course of this study were made on cows receiving similar diets, and the role of the diet is not considered to have influenced the results to any appreciable degree.

Androgen levels in normal cows. To establish a basis for comparison, assays were made of feces of normal dairy cows in various physiological states. Feces of non-pregnant lactating, pregnant lactating and pregnant non-lactating cows produced comb weights 300 to 400 per cent greater than the controls, at the same time suppressing testicular development to 50 to 60 per cent of that of the controls (table 4). The data fail to show a clear-cut difference in androgenic

TABLE 4

Androgen in the feces of normal dairy cows as determined by the chick comb growth response^a

Cow donor	Condition of donor	Sample no.	No. of chicks	Feces consumption per chick	Av. body weight	Av. wt. of testes	Av. comb wt. per 100 g. body wt.
				(g.)	(g.)	(mg.)	(g.)
None	Controls		57		128.5	38.4	73.8
904	Non-pregnant lactating	3	7	16.9	116.7	18.7	322.2
		3	11	17.6	107.4	14.9	290.5
1092	Pregnant lactating ^b	4	10	17.9	112.9	16.7	333.5
		4	9	15.2	103.4	14.7	284.4
1093	Pregnant lactating ^b	5	7	17.2	113.7	18.7	271.7
		5	8	16.7	100.7	19.2	246.1
885	Pregnant non-lactating ^c	8	11	16.6	110.7	21.9	279.4
		8	8	16.4	113.6	17.4	246.5
907	Pregnant non-lactating ^c	9	8	^d	104.0	17.3	241.5
		11	11	17.7	115.6	18.8	385.2
897	Pregnant non-lactating ^c	12	9	18.1	115.5	18.1	325.2
		12	10	17.4	112.4	16.8	282.6

^a The feces were fed to chicks for 14 days as 10% of the ration. Sample numbers indicate where repeat tests were conducted

^b Cows in first 2 months of gestation.

^c Cows in last month of gestation.

^d Data not available.

content of the feces in the different groups. Turner (28) reported that the androgenic activity was high during the first month of lactation but a later report (29) did not substantiate this. Gassner and Longwell (8) report that the androgenic titer drops to zero at parturition. Several reports (8, 18, 28, 29) indicate that the excretion is high shortly before parturition but none specifically states that statistically significant increases were found. In our series, two of three samples collected in late pregnancy did not vary significantly from the mean of all samples collected from normal non-pregnant or early pregnant cows.

Androgen levels in cystic-ovary cows. The etiology of cystic ovaries in cows is as yet unknown. This pathological condition has been studied by several

investigators since the turn of the century (1, 7, 10, 11, 20, 30) but studies on hormonal levels in these animals are not available.

Six cows with a cystic condition of the ovaries were studied during the course of this investigation. A brief history of each case follows:

Cow 1. Jersey, 7 years of age, lactating approximately 2 years. Placed under observation June 22, 1947. Examination on that date disclosed a cyst on the left ovary double the size of a walnut, no cyst on the right ovary. Previous history of frequent mounting and molesting other cows in the herd. Evidence of heat once in 2.5 months under our observation.

Cow D-41. Jersey. Last calf September, 1945. Cyst first discovered November, 1946, shortly after abortion. Cyst expressed for the last time December, 1946. Placed under observation October 28, 1947 and examination revealed the right ovary the size of a small orange, a little larger than the left. Previous history of lowing like a bull and riding all cows available. Used to determine cows in heat. Tail head not significantly raised. During study infrequent riding of companion cow was observed. In heat December 19, 1947. Examination December 20th disclosed a definite cyst, 2×2.5 inches, on the left ovary. Right ovary was normal. Autopsy at end of study showed the right ovary to be 1×1.75 inches with one follicular cyst; the left, 1.75×2 inches with three follicular cysts. No corpora lutea were present.

Cow E-59. Jersey. Last calf June 6, 1946. Obtained for study March 1, 1948. Previous history of nymphomania symptoms, frequent estrous period and breedings, but no conceptions, bellowing, high at the tail head, riding of all cows in heat, pawing and nervousness. No heats observed from March to June, 1948. Examination February 23, 1948, showed the right ovary to be the size of a small hen's egg with a cyst 1 inch in diameter. The left ovary was the size of a large English walnut but had a smooth surface. The ovaries regressed until April 20, when examination showed one cystic follicle on the right ovary. Last examination in June, when the right ovary was the size of an English walnut and had one or more large follicles and the left ovary was small with no palpable follicles or corpora lutea. Cow anaphrodisiac since February, 1948, but still repeatedly mounts cows in heat, is high at the tail head and bellows like a bull.

Cow 216. Last calf June, 1947. Bred every 3 weeks on occurrence of heat between July, 1947, and January, 1948, with no conceptions resulting. No external cystic symptoms except for a slightly raised tail head. Previous history of mounting cows in heat a great deal. Examination in February, 1948, revealed the right ovary inactive, the left ovary half the size of a hen's egg and definitely cystic. Examination again in March disclosed no cysts on either ovary. In heat March 1, 1948, and bred March 2.

Cow 849. Jersey. Last calf December 11, 1947. Bred four times within a 2-month period since her last calf. History of cyst first being noticed March 24, 1948, on the right ovary; left ovary normal. Placed under observation May 11, 1948. Previous observations indicate she frequently was in heat, riding other cows and standing to be ridden. Between May 11 and June 6, she was in heat every 7 to 9 days and exhibited nymphomania symptoms of being very rabid, riding and being ridden almost constantly while in heat. On March 11, the cyst on the right ovary was 0.75 inch in diameter and quite hard. Examination June 3 revealed both ovaries enlarged, a little larger than an English walnut. Injected with 1000 I.U. equine gonadotrophin June 4, and by June 9 the ovaries were smaller, although the left was still about the size of an English walnut, the right ovary being smaller and flatter with a corpus luteum on one end. In heat June 6, but was not observed in heat again up to June 29, although she still mounted other cows in heat. Estrus noted again July 17, July 20 and July 22.

Cow 758. Jersey. Last calf September 3, 1947. Bred five times between September, 1947, and January, 1948. History of displaying nymphomaniac symptoms. In heat often, every 4 to 5 days. Showed morphological characteristics of a high tail head, relaxed pelvic ligament, and tendency toward coarseness in the shoulders. Did not develop the throaty bawling of a bull. Examination March 23 and March 25, 1948, disclosed the left ovary slightly enlarged with a couple of small cysts present with the right ovary apparently normal.

Feces of the six cows were collected and assayed for androgenic activity (table 5). Feces of cows 1 and D-41 produced comb weights which indicated very little excretion of androgens, while those of the remaining cows showed significant androgen content, although two of them were below that of the normal cows. The results suggest a decrease in fecal androgens in cows with cystic ovaries, although results on cows 849 and 758 might be considered as being in the normal range. As may be noticed in the table, there is an apparent correlation between the fecal androgen level and the length of time since calving. The lowered androgen excretion in cystic-ovary cows with masculine charac-

TABLE 5

Androgen in the feces of cystic-ovary cows as determined by the chick comb-growth response

Donor	Months since last calf	Sample no.	No. of chicks	Av. body weight	Av. wt. of testes	Av. comb per 100 g. body weight	Feces consumption per chick
				(g.)	(mg.)	(mg.)	(g.)
None (controls) ^b			13	329.1	86.8	402.4	.
Cow no. 1 ^b	24	1	15	258.1	51.8	381.4	^c
		1	14	249.2	46.2	543.1	^c
None (controls)			161	128.8	40.2	80.1	.
		2 ^a	9	115.5	28.5	71.2	18.2
		2	10	128.9	34.0	71.7	15.3
D 41	26	6	9	109.8	30.5	105.6	19.7
		6	8	120.0	26.6	100.8	16.7
		17	10	123.4	25.1	244.3	18.5
E 59	33	17	11	125.1	19.6	212.6	18.1
		20	10	107.5	24.6	104.0	16.9
216	8	13	9	112.1	21.8	110.0	15.9
		13	10	129.5	30.3	158.7	19.5
849	5	36	9	137.2	29.2	268.2	20.1
758	6	23	8	111.0	20.0	234.9	16.2

^a The feces were fed to chicks for 14 days as 10% of the ration. Sample numbers indicate where repeat tests were conducted.

^b Results over a 28-day feeding period.

^c Data not available.

teristics and behavior is analogous to the low androgen excretion in the bull as reported by Riley and Hammond (22) and Turner (27). The significance of this decreased excretion of fecal androgens in the male and masculine-like female is not apparent.

Effect of ovariectomy on androgen excretion. It has been demonstrated that the ovary may secrete androgens under certain conditions (3, 12, 13, 14, 15, 17), but to what extent it is involved in the normal production of androgens has not been determined. To investigate the role which the ovary plays as a source of fecal androgens in the cow, bilateral ovariectomy was performed on cow 883. Feces were collected for assay preceding the operation and on the 4th, 10th and 39th days post-operative. The results are shown in table 6. Ovariectomy did not produce a decrease in androgen excretion. In fact, there is an apparent

tomy in the cow is a hazardous and difficult procedure, however, resulting in considerable stress and post-operative discomfort. Very little food or water was consumed by the cow for approximately 72 hours after the operation, and defecations were extremely meager. Furthermore, severe infection was found at the site of the removed adrenal at the time of autopsy 26 days after the operation. One may assume, therefore, that, as a result of stress, the remaining adrenal secreted at a greater rate than did both adrenals preceding the operation. The fact that a marked change in fecal androgens occurred following adrenalectomy in an ovariectomized cow provides strong presumptive evidence that the adrenals play a prominent role in androgen production in this species.

DISCUSSION

One of the first questions confronting one attempting to study the factors modifying the excretion of a given hormone is the accuracy of the assay test to be employed. The data given in table 1 indicate that in most instances a given result can be duplicated with considerable precision. However, in 2 of 18 tests there were statistically significant differences in the mean comb weights of duplicate tests on the same sample. This means that in a few instances there have been variables in the procedure or in the test animals which have not been recognized. This fact serves as a warning in the interpretation of minor differences.

That the nature of the diet has a profound effect upon the 24-hour excretion of fecal androgen is indisputable. In three of five tests the fecal androgen per gram of feces was distinctly higher when cows were fed roughage alone than when they were fed concentrates alone (table 2). In addition, the total feces per 24-hour period was approximately three times greater on roughages than on grain (table 3). Until the specific androgen in cow feces is known, attempts to express androgen excretion in absolute terms are of doubtful value. Nonetheless, the response of the chick comb to dehydroisoandrosterone² was determined during the course of this study. When placed in the chick ration using the same strain of chicks, 4 mg. of this androgen per bird produced a comb weight (average of 10 birds) of 170 mg., and 8 mg. of the hormone, an average comb weight of 318 mg. The responses to the feces of cow 757 (table 2) come closest to simulating these responses. The amount of feces consumed per bird and the 24-hour excretion of feces are known. Assuming that the response to the feces of cow 757 on rolled barley, 187 mg. comb weight, is equivalent to the response to 4 mg. of dehydroisoandrosterone, and further that the response to the feces of cow 757 on alfalfa hay, 461 mg. comb weight, is equivalent to the response to 8 mg. of the hormone, one is able to calculate excretion in terms of dehydroisoandrosterone. The calculation discloses that an equivalent of 212 mg. of dehydroisoandrosterone was excreted daily by cow 757 on rolled barley and 1,286 mg. on alfalfa hay. In other words, the daily androgen excretion was approximately six times greater on roughage than on grain.

² Kindly supplied by Schering Corp., Bloomfield, N. J.

Further studies are necessary before one could speculate on the cause of this difference.

The consistency with which responses of 200 mg. or greater in comb weights were obtained when chicks were fed feces from normal animals (table 4) indicates that lower values may have diagnostic significance. As is seen in table 5, a low androgenic response usually is obtained from cows with cystic ovaries of long standing.

The results clearly show that there is no fall in the fecal androgen level following ovariectomy. Too much emphasis should not be put on the rise observed without further experiments. The significance of the finding that in two trials a distinct rise was obtained after treatment of cow 562 with equine gonadotrophin is tempered by the occurrence of a distinct drop in a trial with cow E-59. The fact that a distinct increase in ovary size was found by rectal palpation in the first animal and no detectable ovarian change in the second may furnish a clue for interpretation.

The distinct rise in androgen excretion obtained by unilateral adrenalectomy scarcely is the result expected, but, nevertheless, it does indicate that the adrenal may be involved in determining the amount of fecal androgen in the cow.

SUMMARY

Studies were made on the fecal androgen excretion by dairy cows in various physiological and pathological states.

The accuracy of the assay method was determined with duplicate tests on 18 samples. Sixteen of the 18 tests showed no statistical difference between the mean comb weights of duplicate assays on the same sample.

In four trials, diet was shown to have a marked effect on the androgenic activity of cow feces; the 24-hour excretion of androgens on a roughage diet (alfalfa hay and straw, or alfalfa hay alone) was three to four times greater than on a diet of concentrates alone. The androgenic activity per gram of feces and the total feces excreted in 24 hours both were greater on roughage than on concentrates.

The feces of normal cows, incorporated as 10 per cent of a chick diet, produced an average comb weight per 100 g. body weight of 295.2 as compared to an average comb weight of 80.1 mg. for control chicks receiving no feces. In the small number of animals studied, there was no indication that the fecal androgen level was influenced significantly by lactation or stage of gestation.

The feces of cows with cystic ovaries contained less androgenic activity than the feces of normal animals in four of six cases. In fact, in two cases no evidence of androgen could be detected by the method employed. Thus, it appears that in certain reproductive abnormalities, the fecal androgen level is decreased.

Bilateral ovariectomy of a cow caused an apparent increased androgen excretion. Feces collected 4, 10 and 39 days following ovariectomy produced average comb weights per 100 g. body weight of 157.2, 270.6 and 239.4 mg.,

respectively, as compared to the average comb weight of 183.2 mg. produced by feces collected before the operation.

Ovarian stimulation with equine gonadotrophin produced increased excretion of androgen in two experiments and decreased excretion in one experiment.

Unilateral adrenalectomy of an ovariectomized cow was followed by an increase in androgen excretion. Feces collected 3, 7, 11 and 14 days post-operative produced average comb weights per 100 g. body weight of 419.9, 357.5, 318.9 and 468.3 mg., respectively, as compared to 248.0 mg. for feces collected pre-operatively. As the gonads were absent, a plausible explanation of the results obtained is that the remaining adrenal was stimulated to increased secretory activity.

REFERENCES

- (1) ALBRECHTSEN, J. Die Unfruchtbarkeit des Rindes ihre Ursachen und ihre Behandlung. Richard Schoetz, Berlin. Pp. 102-111. 1920.
- (2) COLE, H. H., HART, G. H., AND MILLER, R. F. Studies on the Hormonal Control of Estrous Phenomena in the Anestrous Ewe. *Endocrinology*, 36: 370-380. 1945.
- (3) DOMM, L. V. Observations Concerning Anterior Pituitary-Gonadal Interrelations in the Fowl. Cold Springs Harbor Symposia Quant. Biol., 5: 241-257. 1937.
- (4) DORFMAN, R. I. Studies on the Bioassay of Hormones: The Relative Reactivity of the Comb of Various Breeds of Chicks to Androgens. *Endocrinology*, 42: 7-15. 1948.
- (5) DORFMAN, R. I., HORWITT, B. N., SHIPLEY, R. A., FISH, W. R., AND ABBOTT, W. E. Metabolism of the Steroid Hormones: Studies on 17-Ketosteroids and Androgens. *Endocrinology*, 41: 470-488. 1947.
- (6) FRANK, R. T., KLEMPNER, E., HOLLANDER, F., AND KRISS, B. Detailed Description of Technique for Androgen Assay by the Chick Comb Method. *Endocrinology*, 31: 63-70. 1942.
- (7) FREI, W., AND LUTZ, E. Der heutige Stand der Forschungen über das Oestrushormon und die Nymphomanie des Rindes. *Virchow's Arch.*, 271: 572-622. 1929.
- (8) GASSNER, F. X., AND LONGWELL, B. B. Fecal Excretion of Androgens by the Dairy Cow During Pregnancy. *Federation Proc.*, 6: 109. 1947.
- (9) HAMBLIN, E. C., ROSS, R. A., CUYLER, W. K., BAPTIST, M., AND ASHLEY, C. Studies on the Metabolism of Androgens in Women. *Endocrinology*, 25: 491-508. 1939.
- (10) HART, G. H., TRAUM, J., AND HAYES, F. M. Bovine Infectious Abortion and Associated Diseases of Cattle and Newborn Calves. *Calif. Agr. Expt. Sta. Bull.* 353. Pp. 362-366. 1923.
- (11) HESS, E. Die Sterilität des Rindes, ihre Erkennung und Behandlung. M. & H. Schaper, Hanover. Pp. 16-148. 1920.
- (12) HILL, R. T. Ovaries Secrete Male Hormone. III. Temperature Control of Male Hormone Output by Grafted Ovaries. *Endocrinology*, 21: 633-636. 1937.
- (13) HILL, R. T. Ovaries Secrete Male Hormone. I. Restoration of the Castrate Type Seminal Vesicle and Prostate Glands to Normal by Grafts of Ovaries in Mice. *Endocrinology*, 21: 495-502. 1937.
- (14) HILL, R. T., AND GARDNER, W. U. Maintenance of Accessory Organs by Ovarian Grafts in Castrate Male Mice. *Anat. Record*, 64, Suppl. 3: 21. 1936.
- (15) HILL, R. T., AND STRONG, M. T. Ovaries Secrete Male Hormone. V. A Comparison of some Synthetic Androgens with the Naturally Occurring Androgen in Mice. *Endocrinology*, 27: 79-82. 1940.
- (16) HIRSCHMANN, H. Androgens from the Urine of Ovariectomized Women. *J. Biol. Chem.*, 130: 421-422. 1939.

- (17) KIRSCHBAUM, A., AND PFEIFFER, C. A. Deposition of Melanin in Sparrow Bill Following Local Action of Testosterone Propionate in Alcoholic Solution. *Proc. Soc. Exptl. Biol. Med.*, **46**: 649-651. 1941.
- (18) LONGWELL, B. B., AND GASSNER, F. X. The Fecal Excretion of Androgens by the Pregnant Cow. *Federation Proc.*, **6**: 272. 1947.
- (19) MCGEE, L. C. The Effect of the Injection of a Lipoid Fraction of Bull Testicle in Capons. *Proc. Inst. Med. of Chicago*, **6**: 242. 1927.
- (20) PEARL, R., AND SURFACE, F. M. Sex Studies. VII. The Assumption of Male Secondary Characteristics by a Cow with Cystic Degeneration of the Ovaries. *Maine Agr. Expt. Sta. Bull.* 237. Pp. 65-80. 1915.
- (21) PINCUS, G. Adrenal Cortex Function in Stress. *Ann. N. Y. Acad. Sci.* (In press.)
- (22) RILEY, G. M., AND HAMMOND, J. C. An Androgenic Substance in Feces of Cattle as Demonstrated by Tests on the Chick. *Endocrinology*, **31**: 653-658. 1942.
- (23) SALMON, U. J., AND GEIST, S. H. Effect of Androgens upon Libido in Women. *J. Clin. Endocrinol.* **3**: 235-238. 1943.
- (24) SELYE, H. Textbook of Endocrinology. *Acta Endocrinologica. Univ. of Montreal, Montreal, Canada.* P. 397. 1948.
- (25) TURNER, C. W. Elimination of Male Hormone by Lactating Dairy Cows. *J. Dairy Sci.*, **29**: 525. 1946.
- (26) TURNER, C. W. The Androgenic (Male) Hormone Content of Dairy Cattle Manure. *J. Animal Sci.*, **5**: 400. 1946.
- (27) TURNER, C. W. The Male Hormone Content of Ruminant Manure. *J. Dairy Sci.*, **30**, 1: 1-8. 1947.
- (28) TURNER, C. W. Factors Influencing Male Hormone Content of Cow Manure. *J. Dairy Sci.*, **30**, 8: 579. 1947.
- (29) TURNER, C. W. Some Factors Influencing the Male Hormone Content of Cow Manure. *J. Dairy Sci.*, **31**: 1032-1040. 1948.
- (30) WESTER, J. Eierstock und Ei. Befruchtung und Unfruchtbarkeit bei den Haustieren. R. Schoetz, Berlin. Pp. 62-74. 1921.

SOME EFFECTS OF FEEDING THYROPROTEIN TO DAIRY COWS DURING THEIR FIRST LACTATION

By J. W. THOMAS, L. A. MOORE AND J. F. SYKES

*Bureau of Dairy Industry, Agricultural Research Administration,
United States Department of Agriculture*

During the past several years various investigators have demonstrated that feeding thyroprotein, an iodinated casein, to producing dairy cows results in increased milk production. Reineke and Turner (16) were the first American workers in this field. The results of the early work have been verified and expanded at several experiment stations (1, 3, 6, 10, 13, 15, 25). Seath *et al.* (19) have studied the effect of feeding thyroprotein in a warm climate. The largest experiment was one carried out by Blaxter (4) in England which involved the use of 1,008 cows in farm herds. This investigator has been studying recently the possibility that thyroprotein feeding may have practical application in England if the thyroprotein is fed when milk production is at a seasonal low point during the latter part of the winter, a period of about 2 months (5). The changes that might occur in the composition of the blood and/or milk as a result of feeding thyroprotein to the dairy cow also have been studied (1, 6, 10, 12, 20, 26). A review of a non-scientific nature on feeding of thyroprotein to dairy cattle appeared in the 1943-47 Yearbook of Agriculture (14).

Most of these experiments have been for short periods and, therefore, the data are not adequate to guide the dairyman in feeding thyroprotein to cows over long periods of time. Also, there are no data concerning the effects of feeding thyroprotein for long periods and/or for several lactations on the health and lifetime performance of cows. Therefore, an experiment was set up by the Bureau of Dairy Industry to study the long-time effects of feeding thyroprotein on the health and reproduction of dairy cattle. Some interesting observations have been made from the standpoint of milk production and the results observed during the first lactation are reported here.

EXPERIMENTAL

Eleven first-calf heifers were fed thyroprotein from approximately the 50th day postpartum until 90 days before the next expected parturition. This amounted to an average of 301 days (range 240 to 380) during which the cows received the drug. A group of three control cows (group 1) received the same feed and treatment except that they were not fed thyroprotein.

All cows were fed 7.5 lb. alfalfa and 7.5 lb. timothy hay and 15.0 lb. of corn silage per 1000 lb. of body weight. This roughage allowance was calculated in early lactation and maintained at that level during the lactation. The remainder of the nutrient allotment of each cow consisted of a 17 per cent protein grain mixture of wheat bran, 40 parts; ground yellow corn, 40 parts; linseed meal, 20 parts; and salt (non-iodized), 1 part.

Received for publication October 11, 1948.

Three levels of thyroprotein feeding were used with cows of group 2. Cows H-268, H-269, J-488 and J-489 were fed 1.5 g. per 100 lb. of body weight. Cows H-262, J-487, J-490, J-496 and H-266 were fed 1.0 g. per 100 lb. and cows J-604 and H-272 were fed 0.6 g. per 100 lb. The letter H before the number indicates the cow was of the Holstein breed; likewise, J indicates Jersey. During the experiment the amount of thyroprotein was adjusted every 10 days according to the cow's body weight. The weighed amount of thyroprotein was mixed with the daily grain allowance.

The cows receiving thyroprotein (group 2) were divided into three subgroups on the basis of total digestible nutrient (T.D.N.) intake. Group 2-A was fed the maximum Morrison requirements based on production and maintenance, as was group 1 (controls). The amount fed to group 2-B was increased after mid-lactation from 100 per cent to either 125 or 150 per cent of requirements and group 2-C was fed 125 per cent of the Morrison requirements throughout the period when thyroprotein was fed. This extra amount of nutrients was not always consumed during the early part of lactation. However, by the 100-150th day of lactation the cows readily consumed the 125 per cent and even 150 per cent when it was offered. The extra allowance of nutrients was fed only during the period thyroprotein was being fed.

Adjustments in the amount of grain fed were made at 10-day intervals and were based on the cow's body weight and milk production at similar intervals. In late lactation the grain allowance was not decreased below a minimum of 0.5 lb. per 100 lb. of body weight. This allowance was continued during the dry period. Thus toward the end of the lactation of groups 1 and 2-A and during the dry period of all cows the T.D.N. consumption was somewhat larger than requirements. Daily feed records of amounts fed and refused served as a basis for calculating T.D.N. consumption of each cow. Composite milk samples were obtained for butterfat test (Babcock) on the 5th and 6th days of each 10-day period.

RESULTS

Initial response and body weight changes. When thyroprotein was fed, all 11 cows responded with an increase in F.C.M. production. The degree of response was variable, some cows showing a considerable increase while others gave a negligible increase. Low responses were given by cows H-269 and J-604, but the increase was transient and too small to show when the production was averaged by 30-day periods. The low rate of thyroprotein feeding (0.6 g. per hundred-weight) to cow J-604 may have been one reason for the small response of this animal. The greatest response was given by cow J-489. This cow's average daily F.C.M. production for the month after thyroprotein feeding was initiated was 8.5 lb. (32 per cent) above her pre-experimental level.

The average daily milk production for the month previous to feeding thyroprotein and the first, second, third and fourth months after are as follows: Groups 2-A and B: 33.6, 36.6, 33.0, 28.5 and 24.3; Group 2-C: 34.3, 36.5, 37.5, 35.6 and 33.3. Corresponding figures for the control cows were 39.0, 35.6,

31.1, 27.9 and 25.9. In figure 1 the average milk production of these groups is graphically presented by 30-day intervals expressed as a percentage of the pre-experimental month.

The cows fed thyroprotein increased an average of 8 per cent (2.8 lb.) in daily milk production during the first month after thyroprotein feeding was started as compared to the pre-experimental month. However, by the fourth and fifth months after thyroprotein feeding was started, the production of the cows in groups 2-A and 2-B (both receiving T.D.N. at 100 per cent of requirement to this time) had decreased on a percentage basis to or below the production of the control cows. Thus after the initial increase in milk production the rate of decline was faster for cows fed thyroprotein and T.D.N. at requirement than it was for the control cows. A very different type of response was ob-

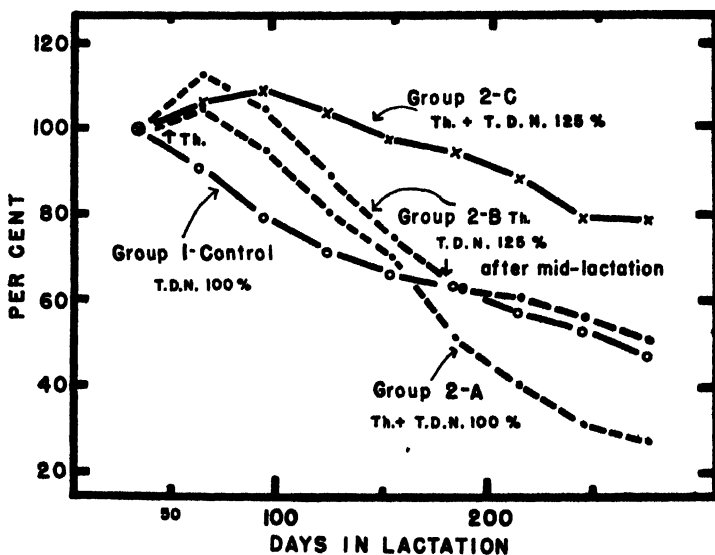


FIG. 1. Effects of feeding thyroprotein with different T.D.N. intakes on the average milk production expressed as a percentage of the pre-experimental month.

tained with the cows in group 2-C, which were given thyroprotein with extra T.D.N. at 50 days postpartum. The peak of response when extra nutrients were fed did not occur until the second or third month after thyroprotein feeding was begun, whereas cows receiving only 100 per cent of their T.D.N. requirement reached their peak within the first month after thyroprotein feeding was begun. Groups 2-A and B, after 4 to 5 months of their regime, had declined in milk production to about 65 per cent of their pre-experimental level, while at the same time the cows that received extra nutrients were producing at about 96 per cent of their pre-experimental level. Thus milk production, after being stimulated by thyroprotein, did not decline as rapidly in the group receiving thyroprotein and extra nutrients as it did in the group receiving thyroprotein and nutrients at 100 per cent of requirement.

When thyroprotein was fed to cows receiving nutrients at 100 per cent of their requirement (groups 2-A and 2-B) large losses in body weight occurred. These losses continued until late in the lactation. Over a corresponding lactation segment the control cows usually gained weight or had small decreases. The average body weight changes from the 50th to the 170th day were as follows: Group 1, a gain of 11 lb.; groups 2-A and B, a decrease of 78 lb. and group 2-C, a gain of 5 lb. The animals fed thyroprotein and extra nutrients from the 50th day had normal body weight changes (group 2-C). Graves (9) reported that well-fed first-calf heifers did not lose weight but usually made small gains after the first month postpartum. This was observed in the control and extra nutrient groups. Thus the feeding of extra nutrients with the thyroprotein prevented the large weight losses noted in animals receiving thyroprotein and nutrients at 100 per cent of requirement.

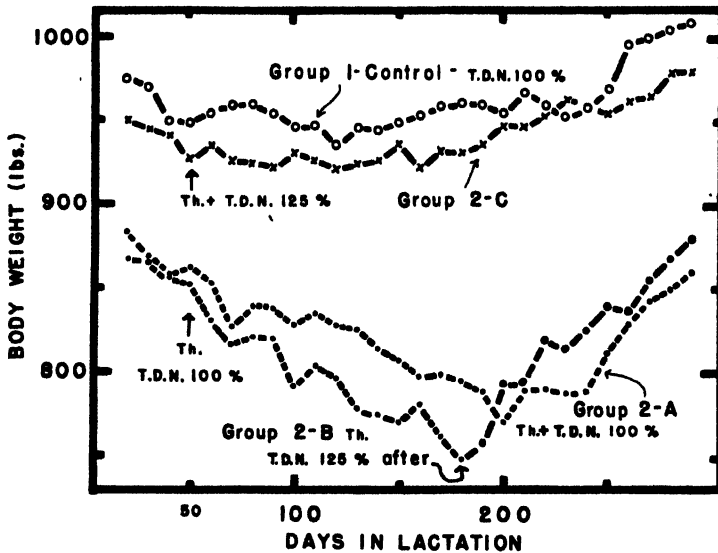


FIG. 2. Effects of feeding thyroprotein with different T.D.N. intakes on the average body weights of the experimental animals.

Effect of increasing T.D.N. intake at mid-lactation. An attempt was made to retard the rapid decreases in body weight and milk production of some cows (group 2-B) that had been receiving thyroprotein and T.D.N. at requirement. This was done by increasing the nutrient intake to 125 or 150 per cent of requirement by feeding additional grain after mid-lactation (at approximately 180 days) to four of the thyroprotein-fed cows. The effects of this treatment on their milk production, body weight and pulse rates can be observed by comparing group 2-A with group 2-B in figures 1, 2 and 3.

Figure 1 shows that the extra nutrients fed to group 2-B beginning at mid-lactation checked the abnormally rapid rate of decline in milk production and from this time on the persistency of this group was comparable to that of nor-

mally-fed cows. However, group 2-A, not fed extra T.D.N., continued to decline in milk production at an abnormally rapid rate. The decrease in daily F.C.M. production of group 2-B during the 2 months following the feeding of extra nutrients averaged only 2.0 lb. A corresponding figure for this segment of lactation of the control group was 3.7 lb., while for group 2-A, not fed extra nutrients, it was 6.4 lb. Throughout the entire lactation the persistency of milk production was excellent for all three first-lactation heifers that received thyroprotein and extra nutrients beginning at 50 days postpartum (group 2-C).

The cows receiving no extra feed (group 2-A) actually weighed less at 240 days in lactation than they did at 180 days (fig. 2). However, the cows in group 2-B, fed extra T.D.N., had gained 75 lb. during this period. The control animals gained an average of 21 lb. Thus the cows that had received thyroprotein for about 120 days and nutrients at 100 per cent of their requirement responded with marked gains in body weight when given extra feed. The body weight of those that did not receive this extra feed continued on a downward trend. However, after the 240th day, rapid increases in body weight occurred in group 2-A due to the fact that milk production had reached a low level and their minimum grain allowance was more than adequate to meet their requirement. When the extra T.D.N. was fed to the cows at mid-lactation, the heart rate showed a marked increase. Variations in heart rate are discussed later in the text. Thus, cows that are receiving thyroprotein and nutrients at 100 per cent of their requirement will respond with increased body weight gains, increased persistency in milk production and increased heart rates when given nutrients at 125 per cent of requirement.

When thyroprotein was removed from the ration at 90 days before the next expected parturition the body weight of all cows increased very rapidly. The increase in body weight averaged about 70 lb. during the first 20 days after the removal of thyroprotein. This rapid gain in body weight and the general appearance of the animal suggests a possible change in water retention when thyroprotein is fed.

The 305-day production records of 14 first-lactation heifers are presented in table 1. They are divided into one control group and three experimental groups that received thyroprotein beginning at about the 50th day of lactation and continued throughout the lactation period. The T.D.N. consumed varied in the three experimental groups.

No definite conclusions can be made concerning total production as affected by the experimental procedures involved because of the small number of animals and the inability to balance inherited productive capacity among the groups. The indications are that the total production for the lactation of cows fed thyroprotein with T.D.N. at requirement would be no greater than cows fed T.D.N. at requirement without thyroprotein. However, the total production of cows H-269, J-488, and J-604 was very low. The conclusion seems justified that poor producing cows cannot be changed into good producers by feeding thyroprotein.

Comparative efficiency of milk production. The comparative efficiency of milk production by the control group and thyroprotein-fed groups is presented in table 2. The lactation segment from the day thyroprotein feeding was started to the 305th day of lactation was used for this comparison. Two animals (J-604 and H-272) that were fed thyroprotein at the low rate were excluded from these calculations.

The F.C.M. produced, the T.D.N. consumed and weight gained during this period were obtained from the records. The T.D.N. assigned to maintenance during this period was calculated by summation of 10-day maintenance requirements based on body weight and Morrison's standard. The T.D.N. for weight gain was calculated by using the figure 3.53 lb. T.D.N. per lb. gain (17).

TABLE 1

305-day production records of 11 cows that have finished their first lactations on the thyroprotein experiment

Cow no. and breed	Rate of thyroprotein feeding (g./100 lb.)	Milk (lb.)	Butterfat (lb.)	F.C.M. (lb.)
<i>Group 1. Control cows (fed according to Morrison's maximum standard).</i>				
J-491	0	5,499	286	6,488
J-485	0	5,930	342	7,505
H-263	0	9,625	413	10,007
<i>Group 2-A. Thyroprotein cows (fed according to standard).</i>				
J-604	0.6	5,181	255	5,897
H-268	1.5	6,853	283	6,979
H-269	1.5	4,693	199	4,863
J-487	1.0	6,514	346	7,804
<i>Group 2-B. Thyroprotein cows (fed 125% of standard last 120 days).</i>				
H-262	1.0	12,017	501	12,330
J-488	1.5	5,297	265	6,106
J-489	1.5	5,778	344	7,474
J-490	1.0	7,020	361	8,226
<i>Group 2-C. Thyroprotein cows (fed 125% of standard during time fed thyroprotein).</i>				
J-496	1.0	7,683	425	9,454
H-266	1.0	10,445	464	11,152
H-272	0.6	9,055	347	8,823

The T.D.N. consumed per 100 lb. of F.C.M. produced was calculated. A similar figure which excluded the nutrients used for weight gain was obtained when the T.D.N. assigned to weight gain was subtracted from the total T.D.N. intake. These two figures were used as a measure of gross efficiency for milk production. The net T.D.N. consumed per 100 lb. of 4 per cent F.C.M. produced were calculated by deducting T.D.N. assigned to maintenance and weight gain from the total T.D.N. intake. This figure was used as a measure of net efficiency for milk production. The formulas used in these calculations and the method of calculating efficiency on the caloric basis also are indicated in table 2.

The gross efficiency calculated by excluding only weight changes is probably

the most practical figure for comparing efficiency of milk production. Control cows consumed about 60 lb. (range 58-62) of T.D.N. (excluding weight gain) to produce 100 lb. of 4 per cent F.C.M. The thyroprotein-fed cows consumed

TABLE 2
Efficiency of milk production from the 50th to 305th day of lactation

Cow no.	F.C.M. produced	T.D.N. consumed	Weight gain	T.D.N. for main-tenances ^a	T.D.N. per 100 lb. F.C.M.		
					Total ^b	Corrected for weight gain ^c	Corrected for gain & main-tenanced ^d
50th to 305th day of lactation							
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
<i>Control cows—group 1.</i>							
H-263	7,650	4,745	85	2,311	62.0	58.1	27.9
J-485	5,472	3,640	68	1,678	66.6	62.1	31.5
J-491	5,036	3,134	50	1,539	62.2	58.7	28.2
Av.	6,053	3,840	68	1,843	63.6	59.6	29.2
Caloric efficiency ^e —29.4%						31.4%	64.0%
<i>Cows fed thyroprotein and T.D.N. at 100%—group 2-A.</i>							
H-268	5,380	4,092	11	1,930	76.1	75.3	39.5
H-269	3,013	3,008	80	1,711	99.9	90.5	33.7
J-487	6,014	3,555	21	1,461	59.1	57.9	33.6
Av.	4,802	3,552	37	1,701	78.4	74.6	35.6
Caloric efficiency ^e —23.8%						25.1%	53.0%
<i>Group 2-B. Cows fed thyroprotein and T.D.N. at 125% during the last 180 days.</i>							
H-262	9,536	5,895	59	2,178	61.8	59.6	36.8
J-488	5,123	3,609	0	1,460	70.4	70.4	41.8
J-489	6,106	4,352	72	1,593	71.3	67.1	41.0
J-490	6,690	3,802	21	1,475	56.8	55.7	33.7
Av.	6,864	4,415	38	1,676	65.1	63.2	38.3
Caloric efficiency ^e —28.7%						29.6%	48.9%
<i>Group 2-C. Cows fed thyroprotein and T.D.N. at 125% during this period.</i>							
H-266	9,267	6,014	33	2,056	64.9	63.6	41.5
J-496	7,917	4,725	38	1,514	59.7	58.0	38.9
Av.	8,592	5,370	36	1,785	62.3	60.8	40.2
Caloric efficiency ^e —30.0%						30.8%	46.6%

^a Obtained by summation of maintenance requirements calculated every ten days.

^b $\frac{\text{Column 3} \times 100}{\text{column 2}}$

^c $\frac{(\text{Column 3}) - (3.53 \text{ column 4}) \times 100}{\text{column 2}}$

^d $\frac{(\text{Column 3}) - (3.53 \text{ column 4} + \text{column 5}) \times 100}{\text{column 2}}$

^e Caloric efficiency = $\frac{340 \text{ F.C.M.} \times 100}{1814 \text{ T.D.N.}} = \frac{1870}{\text{column 6, 7, or 8.}}$

about 65 lb. (range 56-90) of T.D.N. to produce 100 lb. of 4 per cent F.C.M. On a caloric basis, the control cows showed a gross efficiency of 31 per cent.

The average for all thyroprotein-fed cows was 28.1 per cent which was not significantly different from that of the control cows. Neither figure was significantly different from the value of 30 per cent as given by Brody (8). The results of this experiment indicate that feeding a cow thyroprotein does not change significantly gross efficiency for milk production.

Net efficiency for milk production has been shown to decrease as the level of feeding increases, but gross efficiency is essentially independent of level of feeding (8, 11). For this reason it is not valid to compare the values obtained for net efficiency of cows fed extra nutrients (groups 2-B and 2-C) with the cows fed at requirement (groups 1 and 2-A).

All cows in this experiment had values for net efficiency in agreement with the values reported for normal cows fed at their respective levels of T.D.N. intake (8). This indicates that the net efficiency for milk production based on

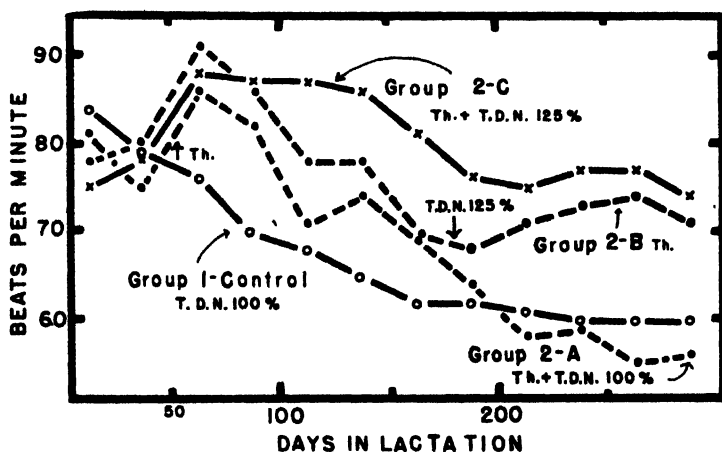


FIG. 3. Effects of feeding thyroprotein with different T.D.N. intakes on the average heart rates of the experimental animals.

the small number of cows fed thyroprotein in this experiment is essentially the same as that of normal cows fed at the same level of T.D.N. intake.

Effect on heart rate. The average heart rate of each group in relation to stage of lactation is presented in figure 3. Heart rates were taken with a stethoscope placed on the chest wall in the region of the heart. The rates were observed once every 5 to 10 days, but are presented in the graph as averages by 25-day intervals.

All cows had heart rates near 80 beats per minute during the first 50 days of lactation. Then the heart rate normally decreased to near 60 at mid-lactation and stayed near this level for the remainder of the lactation. The heart rate increased an average of about ten beats per minute during the first 25 days after thyroprotein was fed. The heart rate then decreased at an abnormally rapid rate in the cows that were fed thyroprotein and T.D.N. at requirement (groups 2-A and 2-B). However, the heart rate increase was sustained until

mid-lactation in the cows fed extra T.D.N. simultaneously with the thyroprotein and then decreased gradually. By mid-lactation the heart rate of cows fed thyroprotein and T.D.N. at requirement had decreased considerably and approached the value for normal cows. After mid-lactation the cows that remained on thyroprotein and T.D.N. at requirement (group 2-A) had heart rates that were similar to those of normal cows. However, when T.D.N. was fed at 125 to 150 per cent of requirement after mid-lactation (group 2-B) the heart rate was increased markedly and the increase persisted. Sykes *et al.* (21) and Ritzman and Benedict (18) have shown that heart rate was affected by level of T.D.N. intake.

Effect on butterfat percentage. The butterfat tests by 10-day periods for

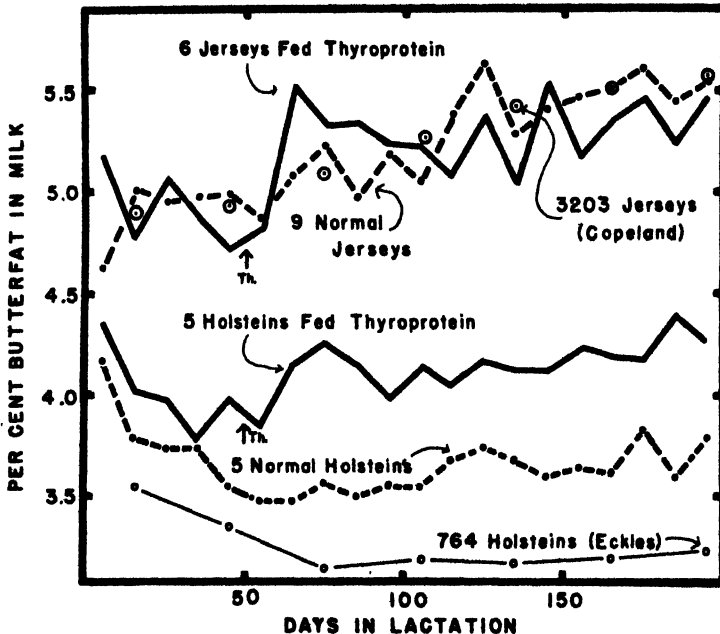


FIG. 4. Effects of feeding thyroprotein on the butterfat percentages and some values from the literature.

the control group and experimental groups for Jerseys and Holsteins, and also some values from the literature (23), are presented in figure 4. Since there were few control animals in the thyroprotein experiment, butterfat tests were obtained from other first-lactation heifers in the herd that had received the same ration. This provided a larger group of control cows in each breed for comparison with the cows fed thyroprotein. Data obtained by Copeland from monthly Jersey Register of Merit records as cited by Turner (23) were considered a reliable standard with which to compare our butterfat tests. The tendency toward an increase in butterfat percentage as lactation progresses is evident in Copeland's data and all our groups (fig. 4).

Butterfat percentages given in the literature for Holstein cows are much lower than the percentages for the experimental and some control Holstein cows in our herd. In past years the Holstein heifers in our herd have had lower butterfat percentages than the heifers in the herd at the present time. Hence the values for the standard and control Holstein group are inherently lower throughout the entire lactation than for the Holstein cows fed thyroprotein.

Soon after thyroprotein feeding was started, there was a marked increase in the mean fat percentage in the milk of the experimental Jersey and Holstein cows. Average butterfat tests during the month before thyroprotein took effect were compared with those during the month afterwards. As judged by the "t" test they showed a highly significant (1 per cent level) increase after the drug was fed in both the experimental Jersey and Holstein groups. No other month-to-month changes in butterfat tests were significant except the decrease from the first to the second month in both the control and experimental Holstein groups. Comparisons of mean butterfat tests for each monthly period between control and experimental Jersey groups showed no significant difference by the "t" test at any month of lactation. Comparing the mean monthly butterfat tests of the experimental Jerseys with those taken from the data of Copeland (23) for R. of M. Jerseys showed no difference except for a significant increase (5 per cent level) for the first month of thyroprotein feeding.

Comparing the mean monthly butterfat tests of experimental and control Holstein groups showed that the increase due to thyroprotein feeding was significant for only the first month of feeding. The results show that a significant increase in butterfat percentage was observed during the first 30 to 40 days of thyroprotein feeding. After about a month of thyroprotein feeding, butterfat tests of thyroprotein-fed cows were not significantly different from those of normally fed cows.

Rate of thyroprotein feeding. Our data do not indicate much difference in the amount of stimulated milk production caused by feeding thyroprotein at either 1.0 or 1.5 g. per 100 lb. body weight. The amount of response appears to be limited more by the individuality of the cow than by the difference in these two rates of thyroprotein feeding. It is possible that with a larger or more homogenous group of cows a better estimate of response in respect to dosage may be obtained.

Cow J-604, when fed thyroprotein at 0.6 g. per 100 lb., showed very little or no stimulation in milk production and in heart rate. However, cow H-272, fed thyroprotein at this same rate but with extra nutrients, showed some stimulation in milk production and in heart rate after the drug was fed. The difference in response of these two cows to the drug may have been due to individual variability of the cows and/or differences in feed intake.

It appears that feeding thyroprotein at the rate of 0.6 g. per 100 lb. is too low to elicit a marked response with some cows. Whether smaller amounts than 1.0 g. per 100 lb. can produce a desirable stimulation without the feeding of extra nutrients is still under investigation.

DISCUSSION

These experiments have verified the results that others have obtained in short term trials in showing that feeding thyroprotein stimulates milk production in most cows. In addition, individual cows evidently differ widely in their response to the drug, and cows that were poor producers have not been changed into good producers by feeding them thyroprotein. During the month after thyroprotein feeding was started, the F.C.M. production of the cows that were fed thyroprotein was 108 per cent of their pre-experimental level. The corresponding figure for the control cows was 91 per cent. This gives a difference of 17 per cent in the amount of milk produced during the first month of feeding thyroprotein. This increase is comparable to that which other investigators observed in short term trials as a result of feeding thyroprotein (3, 4, 15, 25).

Our experiments show that the period of stimulated milk production due to feeding thyroprotein can be lengthened by simultaneously increasing the feed intake of the cow to approximately 125 per cent of requirement. Also, the excessive decreases in body weight that occur when thyroprotein is fed and the T.D.N. intake is limited to requirement do not occur when the T.D.N. intake is increased to 125 per cent of requirement. The first report from this station (13) and also the report by Hibbs and Krauss (10) pointed out that milk-production and body-weight changes due to feeding thyroprotein were dependent upon the nutrient intake of the cow.

Our limited data on total milk production for the entire lactation do not answer the question of whether cows fed thyroprotein and T.D.N. at requirement will produce more than those not fed the drug. Indications are that total production for the lactation period would not be changed markedly as a result of feeding thyroprotein under these conditions. However, feeding T.D.N. at 125 per cent of requirement simultaneously with thyroprotein appears to have resulted in increased production for the first lactation. Whether these cows will continue to produce increased amounts of milk during subsequent lactations is under investigation. The amount of stimulation obtained by feeding control cows T.D.N. at 125 per cent of requirement was not investigated during the first lactation.

One cannot help noticing the similarity between the trends in milk production (fig. 1) and heart rate (fig. 3). In these experiments the responses observed in heart rate and in milk production are similar. The results indicate that heart rate and level of milk production are functions of stage of lactation, T.D.N. intake and thyroid stimulation.

Booth *et al.* (6) failed to find a relation between nutrient intake and heart rate, which is not in agreement with our data or the data of others (18, 21). However, the actual T.D.N. intake of the cows used by Booth *et al.* was not determined. Also, it has been shown that variations in heart rate may be produced by technics used in determining rate, stage of pregnancy, the drying-off process and other factors (2, 22). These facts could explain why Booth *et al.* (6) found no relation between heart rate and level of concentrate feeding.

Thyroprotein administration has been reported (1, 3, 4, 6, 10) to have incon-

sistent effects on the butterfat percentage of the milk. Some cows have shown increases, while others showed no change in butterfat percentage as a result of receiving thyroprotein. Others (15, 16, 19, 20, 25) have stated that thyroprotein or thyroxine administration increased the fat percentage of the milk. In this experiment about 50 per cent of the first-lactation animals showed a marked increase in butterfat percentage after thyroprotein was fed. No animal showed a decrease in butterfat percentage. Evidently feeding thyroprotein increases the butterfat percentage in the milk of some cows and not of others.

However, in comparing the butterfat tests of the cows that were fed thyroprotein with tests published in the literature and with tests of our limited control group, no indication was found that thyroprotein feeding had any prolonged effect on fat percentage. According to our data, after the first 30-40 days of thyroprotein feeding there are no marked differences in butterfat tests between the cows fed thyroprotein and control cows. It appears that the butterfat test of thyroprotein-fed animals is increased only temporarily over that of normal or control animals.

The gross efficiency for milk production of cows with and without thyroprotein administration was within the normal range and near the average value of 30 per cent as given by Brody (8). Brody further stated that the net efficiency for milk production was about 60 per cent when cows were fed T.D.N. at requirement. However, cows fed T.D.N. at higher levels have a lowered net efficiency for milk production. The values for net efficiency obtained with or without thyroprotein administration are similar to the values cited by Brody for corresponding T.D.N. intakes. Using our data to calculate the efficiency of milk production, the results indicate that feeding thyroprotein has not changed the over-all efficiency. Whether efficiency of the various metabolic processes has been altered cannot be determined from our data. It is interesting to note that workers (7, 24) have fed thyroprotein to pigs and found that efficiency of gain was reduced whenever thyroprotein was fed in amounts that would change the rate of gain. The decreased body weight gains and losses in body weight when steers and calves were fed thyroprotein (21, 22) and the data on pigs might indicate that the maintenance requirements of these animals were increased as a result of the thyroprotein feeding. One can only speculate whether feeding thyroprotein to the cow with the resulting increase in heart and respiration rates tends to change the maintenance requirement of the milking cow without altering the over-all efficiency for milk production.

From a practical viewpoint, efficiency is not altered by feeding thyroprotein at the rates used in this experiment. The cows in this experiment consumed about 60 lb. of T.D.N. to produce 100 lb. of 4 per cent F.C.M. whether or not thyroprotein was fed. The data indicate that normal input-output relationships were not disturbed, at least in the first lactation. However, the economy of feeding thyroprotein would depend on the relative cost of the increased nutrients and thyroprotein and the relation of these costs to the value of the increased milk production. More extensive experimental work on this phase of thyroprotein feeding is needed.

For the practical dairyman there are other important factors to consider. The effects that thyroprotein feeding might have on production in succeeding lactations and on the lifetime performance of the cow are undetermined. The effects on reproductive performance, resistance to disease and possible pre- or post-natal effects on the calf must be considered. These aspects will be considered in future reports from this station.

SUMMARY AND CONCLUSIONS

Thyroprotein has been fed to a group of 11 cows for the first lactation beginning 50 days post-partum and continuing an average of 300 days. The following observations have been made:

1. Extra feed must be fed in order to maintain body weight and milk production.

2. If extra feed is not fed, the cows show a period of stimulated milk production followed by a rapid decline in both body weight and milk production.

3. When extra feed (25 per cent above requirement) and thyroprotein were fed simultaneously, high production was maintained throughout the lactation period.

4. The gross efficiency for milk production apparently was unaltered by feeding thyroprotein. Also the net efficiency for the corresponding level of nutrient intake was unchanged.

5. The fat percentage in the milk of thyroprotein-fed cows was increased above that of normally-fed cows for a period of only 30 to 40 days.

6. Heart rate was increased by feeding thyroprotein. The increase was sustained throughout lactation when T.D.N. was fed at 125 per cent of requirement, but the heart rate was increased temporarily, then decreased rapidly if T.D.N. were fed at requirement. It was pointed out that heart rate, like milk production, definitely was related to level of T.D.N. intake, stage of lactation, and thyroprotein administration.

7. Cows that are poor producers respond less than cows that are good producers, so it is not possible to make a good cow from a poor cow by administering thyroprotein.

8. Since all the effects of feeding thyroprotein to producing dairy cows have not been determined at this time there is no sound basis on which one can make recommendations to the practical dairyman concerning the advisability of feeding thyroprotein.

REFERENCES

- (1) ARCHIBALD, J. G. Some Effects of Thyroprotein on the Composition of Milk. *J. Dairy Sci.*, 28: 941-947. 1945.
- (2) BLAXTER, K. L. The Normal Variation in the Heart Rate of Dairy Cows. *Vet. J.*, 99: 2-4. 1943.
- (3) BLAXTER, K. L. The Preparation and Biological Effects of Iodinated Proteins: The Effect of Iodinated Protein Feeding on the Lactating Cow. *J. Endocrinol.* 4: 237-299. 1945.
- (4) BLAXTER, K. L. Experiments with Iodinated Casein on Farms in England and Wales. *J. Agr. Sci.*, 36: 117-150. 1946.

- (5) BLAXTER, K. L. Personal Communication. Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland. 1947.
- (6) BOOTH, A. N., ELVEHJEM, C. A., AND HART, E. B. Some Effects of Feeding Iodinated Casein to Dairy Cows. *J. Dairy Sci.*, 30: 443-455. 1947.
- (7) BRAUDE, R. The Effect of Feeding Iodinated Casein to Pigs. *J. Agr. Sci.*, 37: 45-50. 1947.
- (8) BRODY, SAMUEL. Bioenergetics and Growth. Reinhold Publishing Corp., New York, N. Y. 1945.
- (9) GRAVES, R. R. Can Good Producing Cows Be Fed in Such Manner as to Maintain Their Weight? *J. Dairy Sci.*, 30: 41-47. 1947.
- (10) HIBBS, J. W., AND KRAUSS, W. E. The Effect of Thyroprotein (Protamone) on Milk Production and on Some of the Constituents of the Milk and Blood of Dairy Cows. *J. Animal Sci.*, 6: 162-173. 1947.
- (11) JENSEN, E., KLEIN, J. W., RAUCHENSTEIN, E., WOODWARD, T. E., AND SMITH, R. H. Input-output Relationships in Milk Production. U. S. Dept. Agr. Tech. Bull. 815. 1942.
- (12) KEMMEER, A. R., BOLOMEY, R. A., VAVICH, M. G., AND DAVIS, R. N. The Effect of Thyroprotein upon Vitamin Content of Milk. *Proc. Soc. Exptl. Biol. Med.*, 63: 309-310. 1946.
- (13) MOORE, L. A. Some Physiological Effects of Feeding Thyroprotein to Dairy Cattle. *J. Dairy Sci.*, 29: 532. 1946.
- (14) MOORE, L. A., AND SYKES, J. F. Thyroprotein for Cows. U. S. Dept. of Agr. Yearbook, 1943-47: 107-112. 1947.
- (15) REECE, R. P. The Influence of a Synthetic Thyroprotein when Fed to Dairy Cows Over an Extended Period. *J. Dairy Sci.*, 30: 313-324. 1947.
- (16) REINEKE, E. P., AND TURNER, C. W. Increased Milk and Milk Fat Production Following the Feeding of Artificially-Formed Thyroprotein. *J. Dairy Sci.*, 25: 393-400. 1942.
- (17) Report of Joint Pasture Committee of Am. Soc. Agronomy, Am. Dairy Sci. Assoc., and Am. Soc. Animal Production. Preliminary Report on Pasture Investigations Technique. *J. Dairy Sci.*, 26: 353-369. 1943.
- (18) RITZMAN, E. G., AND BENEDICT, F. G. The Effect of Varying Feed Levels on the Physiological Economy of Steers. New Hampshire Agr. Expt. Sta. Tech. Bull. 26. 1924.
- (19) SEATH, D. M., BRANTON, C., AND GROTH, A. H. Effect of Feeding Iodinated Casein on Production and Health of Milking Cows. *J. Dairy Sci.*, 28: 509-517. 1945.
- (20) SMITH, J. A. B., AND DASTUR, N. N. Studies on the Secretion of Milk Fat. 3. The Effect of Thyroxine Administration on the Blood Lipoids and on the Nature of the Milk Fat. *Biochem. J.*, 34: 1093-1107. 1940.
- (21) SYKES, J. F., WRENN, T. R., MOORE, L. A., AND THOMAS, J. W. The Effect of Energy Intake on Heart Rate in Hyperthyroidism Induced by Feeding Thyroprotein. *Am. J. Physiol.*, 153: 412-416. 1948.
- (22) THOMAS, J. W., MOORE, L. A., AND SYKES, J. F. Unpublished data. 1947-48.
- (23) TURNER, C. W. Factors Affecting the Composition of Milk. Mo. Agr. Expt. Sta. Bull. 365. 1936.
- (24) VANDER NOOT, G. W., REECE, R. P., AND SKELLEY, W. C. Effects of Thyroprotein and of Thiouracil Alone and in Sequence in the Ration of Swine. *J. Animal Sci.*, 7: 84-91. 1948.
- (25) VAN LANDINGHAM, A. H., HENDERSON, H. O., AND WEAKLEY, C. E., JR. The Effect of Iodinated Casein (Protamone) on Milk and Butterfat Production and on the Ascorbic Acid Content of the Milk. *J. Dairy Sci.*, 27: 385-396. 1944.
- (26) VAN LANDINGHAM, A. H., HYATT, G., JR., AND WEAKLEY, C. E., JR. Effect of Feeding Iodinated Casein to Dairy Cows on the Protein Composition and Content of Milk. *J. Dairy Sci.*, 29: 533. 1946.

SEMEN PRODUCTION AND FERTILITY OF DAIRY BULLS FED RATIONS CONTAINING PROTEINS OF PLANT AND ANIMAL ORIGIN

CECIL BRANTON,¹ R. W. BRATTON, AND G. W. SALISBURY²

*Laboratory of Animal Breeding and Artificial Insemination,
Department of Animal Husbandry, Cornell University, Ithaca, New York*

The effect of the source of protein in the rations of mature dairy bulls used in artificial breeding has received little attention in this country (14). However, a number of Russian workers (16, 19, 20, 28, 29) have stressed the necessity of including animal proteins in the rations of stud bulls.

The feeding and management practices in Russia differ considerably from those in general use in this country. Bulls used in studs in Russia in many instances have been fed rations very low in protein (19, 28, 29). Also, their practice (12, 13, 16, 20, 33) has been to use bulls periodically and collect semen from them much more frequently during these periods than has been done in the United States. Furthermore, it appears that only a few bulls have been studied and these for short periods of time by these workers (12, 13, 20, 28). Since the authors have seen only the abstracts of these papers, the experimental designs employed cannot be evaluated.

Although the Russian workers have concluded that the animal proteins contained in blood meal and skim milk caused marked improvement in sperm resistance and concentration and apparently affected spermatogenesis favorably, the authors believe these conclusions are open to question. Bratton (7) found that for dairy cows, protein quality apparently was of little practical importance when the ration consisted of common roughages and concentrates. Tankage, fish meals and dried blood meal have been compared with vegetable protein supplements such as soybean oil meal, cottonseed meal and linseed meal in the ration of lactating dairy cows (2, 3, 4, 17). Milk production was either slightly lower or about equal for the cows fed the animal proteins as compared to the production of the cows fed the protein supplements of vegetable origin. Recent studies (8, 10, 11, 21, 32, 34, 35, 36) have shown that proteins may be synthesized by the bacteria of the rumen from simple nitrogenous compounds such as urea and some ammonium compounds. Such evidence suggests there should be no advantage in feeding animal proteins as compared to vegetable proteins as supplements in the ration of dairy bulls when the level of digestible protein is adequate.

Because the evidence published by the Russian investigators (16, 19, 20, 28, 29) was not conclusive and seemed to be contradicted by the investigations on quality of protein for the dairy cow and experiments involving the synthesis of proteins in the rumen, the experiment reported herein was undertaken. The

Received for publication October 15, 1948.

¹ Now at Louisiana Agricultural Experiment Station, Baton Rouge.

² Now at Department of Dairy Production, University of Illinois, Urbana.

purpose of this investigation was to relate differences in measurable semen characteristics and relative fertility of dairy bulls used routinely in artificial insemination to concentrate mixtures containing primarily either animal or vegetable sources of supplementary protein.

EXPERIMENTAL METHODS AND MATERIALS

Feeds used. Since cereal grains and their by-products are decidedly inferior to such food as milk in the quality of protein contained and since the proteins of corn grain are low in lysine and tryptophane (18), an all-cereal concentrate mixture containing corn gluten feed as the protein supplement was chosen for study. In contrast, skim milk powder, which contains an abundance of protein of exceptionally high quality and which supplements so as to correct the deficiencies in the proteins of the cereal grains, was chosen as an animal source of supplementary protein. Soybean oil meal, though of plant origin, is especially rich in protein, and its protein is of excellent quality (18). Therefore, it was chosen as representative of a good plant protein. Timothy hay was used as the only roughage because it is low in protein, is readily available and is not recommended by Morrison (18) when a feed such as corn gluten feed, corn gluten meal, brewers' dried grains or distillers' dried grains is the protein supplement.

In a previous study reported by the authors (6), it was found that a concentrate mixture containing approximately 12 per cent protein, when fed with mixed hay, supplies sufficient protein for the maintenance of body weights as well as semen production of bulls. However, since timothy hay was selected instead of mixed or legume hay and since comparisons between protein supplements were being made, the concentrate mixtures were made to contain approximately 16 per cent protein (calculated). Also, it was reported by these authors (6) that T. D. N. intakes of approximately 110 per cent of the recommended maintenance requirements for dry dairy cows of equivalent weights (18) were required to maintain the body weights of bulls. The amounts of roughage and concentrates were adjusted to furnish this level of T. D. N. intake, with approximately 40 per cent of the T. D. N. intake furnished by the concentrates.

The ingredients used to make up each of the concentrate mixtures, the chemical analyses of each mixture and that of the hay, and the cost per ton of each concentrate are given in table 1.

The feeding schedule for each bull was based on initial body weight. This schedule was not altered as a consequence of subsequent changes in body weight of individual bulls during the three experimental periods.

Management of bulls. All bulls were exercised daily in a small dry lot or on a mechanical exerciser. The latter exercise was equivalent to a slow walk for approximately 1 mile. Throughout the experiment, each bull was used for service approximately once every 13 days, at which time one, two or three ejaculates were collected. Occasionally a fourth ejaculate was collected.

The experimental design. For the study of the three protein supplements, a "double change over" design, as described by Cochran *et al.* (9), involving

three 120-day periods, was employed. One Ayrshire, eleven Holstein-Friesian, and six Guernsey bulls, averaging 7.64 ± 0.44^s years of age and 1866 ± 67^s lb. in body weight, were selected from the stud of the New York Artificial Breeders' Cooperative, Inc., on the basis of their previous semen production and fertility records and the likelihood that they would remain in active service throughout the 360-day experiment. Assignment to the six groups was made on the basis of information available concerning each bull's semen production, fertility and body weight. The assignment of the sequences of the concentrate mixtures A, B and C to each bull was done in the manner described by Cochran *et al.* (9).

TABLE 1

Ingredients and chemical composition of the concentrate mixtures and hay

	Concentrate mixtures			Hay timothy
	A	B	C	
Ingredients	(lb.)	(lb.)	(lb.)	
Hominy feed	635	760	820	
Ground oats	500	610	660	
Wheat bran	200	200	200	
Soybean oil meal			280	
Skim milk powder		390		
Corn gluten feed	625			
Steamed bone meal	20	20	20	
Salt	20	20	20	
Total	2000	2000	2000	
Chemical composition^a	(%)	(%)	(%)	(%)
Moisture	10.49	10.16	14.46	7.76
Crude protein	14.00	14.78	14.73	6.03
Ether extract	3.69	3.12	2.08	1.46
Crude fiber	8.87	6.12	8.17	39.23
Nitrogen-free extract	57.15	60.06	55.35	41.77
Ash	5.80	5.76	5.21	3.75
Total	100.00	100.00	100.00	100.00
Total digestible nutrients (%)^b ..	76.13	77.60	77.05	51.60
Cost per ton (dollars)^c ..	70.60	120.50	71.20	

^a By analysis.

^b By calculation.

^c Actual cost at time of experiment.

The experimental design employed randomly distributed the seasons of the year throughout the treatments.

Measurements of the results. The following criteria were used to evaluate the semen samples: volume, per cent of motile spermatozoa (5), concentration (numbers of spermatozoa per mm.³) (25), and methylene blue reduction time (37). Each semen sample which met the specifications for use in artificial insemination was diluted with yolk-citrate-sulfanilamide diluent (27) and shipped by the Cooperative to its affiliated local units throughout New York State for

^s Standard error of mean.

use in inseminating cows. Semen samples which did not meet the specifications for shipment were discarded. This fact itself was used as an additional over-all criterion of semen quality.

The semen dilution rates employed varied from 1:30 to 1:125, depending on the need for semen on any particular day. These varying rates were not confounding factors within the experiment, as has been shown by investigations of Salisbury (24, 26).

The fertility of the semen samples was determined from the number of services to cows being bred artificially for the first time or for the first time after calving and whether or not these cows returned to artificial service during a period of 60 to 90 days. Fertility is expressed as the per cent non-returns to first services.

In order to establish the initial body weight and an indication of body weight changes of each bull, the bulls were weighed on 3 consecutive days at the beginning of the experiment and at the end of each 120-day experimental period.

Data were recorded routinely on the characteristics of each ejaculate of semen produced by each bull at each collection period, on the fertility of the semen used for insemination and on the changes in body weights of the bulls. For the statistical analyses of the semen characteristics, the values for each ejaculate were used as single observations. Since the number of ejaculates per bull varied, the significance of treatment differences was tested after correcting for unequal subclass numbers in the analysis of variance (15, 31).

RESULTS AND DISCUSSION

The results of the investigation are presented in three parts, namely, the characteristics of the semen produced, the fertility of the semen used and the changes in body weights of the bulls.

Characteristics of the semen. The number of ejaculates and the mean values for the different characteristics used in the evaluation of the semen are shown in table 2. These means represent first, second, third and, in a few instances, fourth ejaculates.

The analyses of variance of the data on the semen characteristics revealed that except for significant differences between individual bulls, groups of bulls and experimental periods, all of which were expected, the only significant difference for treatments was between the average volume per ejaculate produced by the bulls on concentrate mixtures *A* or *B* as compared to those on concentrate mixture *C*. Although the average volume of the ejaculates of the bulls on concentrate mixture *C* was significantly lower (at the 1 per cent level of probability) than that of the bulls fed either mixture *A* or *B*, this was not considered to be a difference of any practical importance. The results as shown in table 2 are interpreted to mean that corn gluten feed, skim milk powder or soybean oil meal as protein supplements in the concentrate mixture fed with timothy hay to bulls used routinely in artificial insemination are of equal value for semen production.

TABLE 2
Summary of semen characteristics

Concentrate mixture	Semen characteristics				
	Vol.	Motile sperm	Number of sperm per mm. ² semen	Methylene blue reduction time	Ejaculates shipped
	(ml.)	(%)	($\times 10^3$)	(min.)	(%)
A—Corn gluten feed					
Mean	6.33	72.8	1160	5.75	68.0
Number of ejaculates	253	253	253	229	253
B—Skim milk powder					
Mean	6.39	69.5	1159	5.48	65.2
Number of ejaculates	260	256	258	226	259
C—Soybean oil meal					
Mean	5.85	69.4	1133	5.71	61.3
Number of ejaculates	279	275	277	239	279

These findings are in agreement with those of Reid *et al.* (22, 23) who compared the effect of feeding a simple concentrate mixture containing ground yellow corn, beet pulp, corn gluten meal, cane molasses and iodized salt with that of feeding a complex mixture containing linseed meal and soybean meal as the protein supplements on the semen production of young bulls. These workers (23) report that "regardless of the diet fed, the concentration of spermatozoa in semen, the initial motility, the degree of livability, the size of the spermatozoa, the quantity of total reducing substances, reducing substances in oxidized state, potential reducing capacity, ascorbic acid, and the initial pH of the semen of bulls" from 18 to 33 months of age were similar. However, it should be pointed out that these investigators (22, 23) fed no protein supplement of animal origin, and they were not dealing with mature dairy bulls.

On the other hand, the results reported in this paper do not confirm those of the Russian investigators (16, 19, 20, 28, 29).

Fertility of the semen used. Fertility data were obtained only on those ejaculates meeting the relatively high standards of semen quality required by the New York Artificial Breeders' Cooperative, Inc. These data, presented in summary form in table 3, show, by treatments, the total number of first serv-

TABLE 3
Summary of fertility data

Treatments	Fertility criteria		
	Total first services	Total 60-90-day non-returns	% 60-90-day non-returns
Concentrate mixture:			
A—Corn gluten feed	15,827	10,058	63.5
B—Skim milk powder	16,692	10,285	61.6
C—Soybean oil meal	15,252	10,026	65.7

ices, the total number of 60-90-day non-returns and the calculated per cent non-returns. When the per cent non-returns for each semen sample was used as a single observation, the analyses of the variance showed that the semen used from bulls fed concentrate mixture *C* (soybean oil meal as the protein supplement) gave an average fertility level that was significantly higher at the 1 per cent level of probability than that from the bulls fed concentrate mixture *B* (skim milk powder as the protein supplement). However, the average difference in fertility of the semen from bulls on all three of the concentrate mixtures was small.

The authors have no explanation as to why the fertility of the semen from bulls receiving the concentrate mixture containing skim milk powder as the

TABLE 4
Initial body weights and body weight changes

Bull	Initial body weight	Body weight changes (Initial minus end of period) ^a		
		End of period 1	End of period 2	End of period 3
	(lb.)	(lb.)	(lb.)	(lb.)
1	1898	62	82	- 55
2	1975	65	57	- 82
3	1525	29	101	- 38
4	2094	- 5	74	- 25
5	1959	56	139	- 17
6	1945	- 22	37	
7	2044	96	146	- 12
8	1959	- 23	86	1
9	2013	90	39	- 28
10	2123	5	19	- 89
11	2207	63	109	- 97
12	2441	12	101	
13	1421	64	19	- 19
14	1623	47	79	- 16
15	1559	- 27	19	- 47
16	1695	5	99	18
17	1642	8	113	- 52
18	1458	- 4	84	- 5
Means	1866	29	78	- 35

^a Body weight changes were based on the weight at the beginning of the experiment.

protein supplement was the lowest. Certainly, there appears to be no advantage of feeding skim milk powder as a protein supplement instead of corn gluten feed or soybean oil meal for periods of 120 days to bulls used for artificial breeding when these bulls are receiving timothy hay as the only roughage. Since the cost of the concentrate mixture containing the skim milk powder is almost twice that of either of the other concentrate mixtures (table 1), these findings are of economic importance.

Body weight changes. The initial body weight and body weight changes for individual bulls, and the respective means for all bulls used in this experiment are given in table 4.

As stated previously, an attempt was made in this study to maintain body weights of the bulls by feeding at the rate of 110 per cent of the recommended

T. D. N. requirements for the maintenance of dry dairy cows. As shown in table 4, the body weight changes during periods 1 and 2 were mostly increases, but during period 3, mostly decreases. The reason for the decrease in body weight of practically all the bulls during the last experimental period is not apparent, since the composition of the concentrate mixtures and the quality of the timothy hay fed appeared to be about the same through the experiment. However, the last period was during the warm months, June, July, August, September and October.

SUMMARY

Using 18 bulls, 11 Holstein-Friesians, 6 Guernseys and 1 Ayrshire, studies were conducted relating differences in measurable semen characteristics and relative fertility to concentrate mixtures containing corn gluten feed, skim milk powder or soybean oil meal as the protein supplement when fed with timothy hay as the only roughage.

The results, as judged by the averages for volume of semen per ejaculate, per cent of motile spermatozoa and their rates of motility, the number of spermatozoa per mm.³ of semen, the methylene blue reduction time and the per cent of usable samples during a 120-day period, showed that corn gluten feed, skim milk powder and soybean oil meal were approximately equal in value as protein supplements in the concentrate mixture.

Based on 60 to 90 days non-returns to first service cows, the average fertility levels of the semen produced when the bulls were fed corn gluten feed, skim milk powder and soybean oil meal were 63.5, 61.6 and 65.7 per cent, respectively. While the average per cent non-returns was significantly higher during the periods when soybean oil meal was fed as compared to the periods when skim milk powder was fed, the difference was relatively small and it is doubtful whether any real advantage lies with the soybean oil meal. In these studies, animal protein was not superior to the plant proteins. The monetary economy of the particular plant protein sources used in these trials was much greater than for the animal protein source.

A T. D. N. intake of 110 per cent of Morrison's recommended requirements for the maintenance of dry dairy cows of equivalent weight resulted in consistent body weight increases for all bulls during the first 240 days but consistent decreases during the last 120 days of the experiment. While the decreases occurred during the summer and early fall months, it was not definitely established whether the observed decreases represented a true cause and effect relationship.

ACKNOWLEDGMENT

The assistance and cooperation of the New York Artificial Breeders' Cooperative, Inc., in furnishing and caring for the experimental animals is acknowledged gratefully.

The authors are indebted to R. E. Elliott and M.J. Bell, Laboratory of Animal Nutrition, Department of Animal Husbandry, Cornell University, for the chemical analyses of the feeds.

REFERENCES

- (1) ANDERSON, J. The Semen of Animals and Its Use for Artificial Insemination. Tech. Communication, Imp. Bur. Animal Breeding and Genetics. Edinburgh. 1945.
- (2) ARCHIBALD, J. C. Tankage as a Source of Protein for Dairy Cows. Mass. Agr. Expt. Sta. Bull. 321. 1935.
- (3) ARCHIBALD, J. C. Dried Blood Meal as a Source of Protein for Dairy Cows. Mass. Agr. Expt. Sta. Bull. 334. 1936.
- (4) ATKESON, F. W., CAVE, H. W., AND RIDDELL, W. H. Kansas Agr. Expt. Sta., 8th Biennial Report, p. 68. 1936.
- (5) BECK, G. H., AND SALISBURY, G. W. Rapid Methods for Estimating the Quality of Bull Semen. J. Dairy Sci., 26: 483-494. 1943.
- (6) BRANTON, C., BRATTON, R. W., AND SALISBURY, G. W. Total Digestible Nutrients and Protein Levels for Dairy Bulls Used in Artificial Breeding. J. Dairy Sci., 30: 1003-1013. 1947.
- (7) BRATTON, R. W. Influence of Quality of Protein in the Concentrate Mixture on the Milk Production of Dairy Cows Fed Mixed Hay and Corn Silage as Roughages. Thesis, Cornell Univ. 1942.
- (8) BRIGGS, H. M., GALLUP, W. D., DARLOW, A. E., STEPHENS, D. F., AND KINNEY, C. Urea as an Extender of Protein when Fed to Cattle. J. Animal Sci., 6: 445-460. 1947.
- (9) COCHRAN, W. G., AUTREY, K. M., AND CANNON, C. Y. A Double Changeover Design for Dairy Cattle Feeding Experiments. J. Dairy Sci., 24: 937-951. 1941.
- (10) GALL, L. S. Some Studies on the Rumen Microorganisms of Sheep and Cattle. Thesis, Cornell Univ. 1946.
- (11) JOHNSON, B. C., HAMILTON, T. S., ROBINSON, W. B., AND GAREY, J. C. On the Mechanism of Non-protein Nitrogen Utilization by Ruminants. J. Animal Sci., 3: 287-298. 1944.
- (12) KIRILLOV, V. S. The Sexual Life of the Bull. (Transl. title.) 1st All-Union Conf. Artif. Insem., pp. 142-148. 1935. (Abs., Animal Breeding Abs., 3: 255. 1935.)
- (13) KIRILLOV, V. S., AND MOROZOV, V. A. Standards of Utilization of Stud Bulls. (Transl. title.) Usp. zuotah. Nauk., 4: 140-158. 1939.
- (14) KNOOP, C. E. The Effect of Feeding Potassium Iodide and Skim Milk Powder on Spermatogenesis. J. Dairy Sci., 29: 555. 1946.
- (15) LOVE, H. H. Experimental Methods in Agricultural Research. Agr. Expt. Sta., Univ. of Puerto Rico, Rio Piedras, P. R. 1943.
- (16) MILOVANOV, V. K. On Utilization of Stud Sires. (Transl. title.) Lenin Acad. Agr. Sci., pp. 4-35. 1939. (Abs., Animal Breeding Abs., 8: 210. 1940.)
- (17) MONROE, C. F., KRAUSS, W. E., AND HAYDEN, C. C. Menhaden Fish Meal as a Protein Supplement for Dairy Cows. Ohio Agr. Expt. Sta. Bimonthly Bull. 176. 1935.
- (18) MORRISON, F. B. Feeds and Feeding. 20th ed., unabridged. The Morrison Publishing Co., Ithaca, N. Y. 1938.
- (19) OVBANNIKOV, G. E. Standards for Feeding Stud Bulls. (Transl. title.) Lenin Acad. Agr. Sci., pp. 36-49. 1939. (Abs., Animal Breeding Abs., 8: 242. 1940.)
- (20) PANKEVIC, A. A. The Effect of the Protein Levels of the Diet on the Sperm of Breeding Bulls. (Transl. title.) Lenin Acad. Agr. Sci., pp. 79-93. 1940. (Abs., Animal Breeding Abs., 13: 24-25. 1945.)
- (21) PEARSON, R. M., AND SMYTH, J. A. B. Utilization of Urea in Bovine Rumen. I. Methods of Analysis of Rumen Ingesta and Preliminary Experiments in Vivo. Biochem. J., 37: 142-148. 1943.
- (22) REID, J. T., WARD, G. M., AND SALSBURY, R. L. Simple versus Complex Concentrate Mixtures for Young Breeding Bulls. I. Growth, Blood Composition, and Cost. J. Dairy Sci., 31: 429-438. 1948.
- (23) REID, J. T., WARD, G. M., AND SALSBURY, R. L. Simple versus Complex Concentrate Mixtures for Young Breeding Bulls. II. Semen Production. J. Dairy Sci., 31: 439-447. 1948.

- (24) SALISBURY, G. W. Fertility of Semen Diluted at 1:100. *J. Dairy Sci.*, 29: 695-697. 1946.
- (25) SALISBURY, G. W., BECK, G. H., ELLIOTT, I., AND WILLETT, E. L. Rapid Methods for Estimating the Numbers of Spermatozoa in Bull Semen. *J. Dairy Sci.*, 26: 62-78. 1943.
- (26) SALISBURY, G. W., AND BRATTON, R. W. Fertility Level of Bull Semen Diluted at 1:400 With and Without Sulfanilamide. *J. Dairy Sci.*, 31: 817-822. 1948.
- (27) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (28) SMIRNOV-UGRJUMOV, D. V. The Influence of Diet on the Sexual Activity of Stud Bulls. (Transl. title.) *Probl. Zivotn.*, No. 3, pp. 43-51. 1937. (Abs., *Animal Breeding Abs.*, 5: 401-402. 1937.)
- (29) SMIRNOV-UGRJUMOV, D. V., AND LAPTEV, M. A. The Feeding of Stud Bulls during Mating Season. (Transl. title.) *Probl. Zivotn.*, No. 2, pp. 64-72. 1938. (Abs., *Animal Breeding Abs.*, 6: 293-294. 1938.)
- (30) SMITH, J. A. B., AND BAKER, F. Utilization of Urea in Bovine Rumen. IV. Isolation of Synthesized Material and Correlation Between Protein Synthesis and Microbial Activities. *Biochem. J.*, 38: 496-505. 1944.
- (31) SNEDECOR, G. W. *Statistical Methods*. 4th ed. The Iowa State College Press, Ames. 1946.
- (32) STUDT, E. Über die Menge und Verdaulichkeit des durch zymogene Symbiose in Pansen des Wiederkauers erzeugten Bakterieneiweissen auf Grund von Schlachtergebnissen. *Z. Tierzücht. Zuchtungsbiol.*, 44: 253-261. 1939.
- (33) VEREVKINA, T. P., AND SMIRNOV-UGRJUMOV, D. V. The Activity of Bulls in Natural Mating. *Probl. Zivotn.*, No. 3, pp. 178-179. 1938. (Abs., *Animal Breeding Abs.*, 8: 35. 1940.)
- (34) WEGNER, M. I., BOOTH, A. N., BOHSTEDT, G., AND HART, E. B. The "In Vitro" Conversion of Inorganic Nitrogen to Protein by Microorganisms from the Cow's Rumen. *J. Dairy Sci.*, 23: 1123-1129. 1940.
- (35) WEGNER, M. I., BOOTH, A. N., BOHSTEDT, G., AND HART, E. B. Preliminary Observations on Chemical Changes of Rumen Ingesta With and Without Urea. *J. Dairy Sci.*, 24: 51-56. 1941.
- (36) WEGNER, M. I., BOOTH, A. N., BOHSTEDT, G., AND HART, E. B. The Utilization of Urea by Ruminants as Influenced by the Level of Protein in the Ration. *J. Dairy Sci.*, 24: 835-844. 1941.
- (37) WILLETT, E. L., AND SALISBURY, G. W. The Effect of Various Diluters, Cooling Rate, Temperature of Storage, and Some Other Factors on the Livability of Spermatozoa in Stored Samples of Semen. N. Y. (Cornell) *Agr. Expt. Sta. Mem.* 249. 1942.

A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDROGUAIAR- RETIC ACID IN DAIRY PRODUCTS. III. ITS ANTIOXYGENIC PROPERTIES IN SWEETENED FROZEN CREAM

J. W. STULL, E. O. HERREID, AND P. H. TRACY

Department of Food Technology, University of Illinois, Urbana

Sucrose sometimes is added to cream which is to be stored frozen in order to lessen the undesirable destabilizing effect on the fat emulsion during freezing and thawing (3, 4, 6, 8, 9, 10, 13). Furthermore, some investigators (7, 9, 12) have found that added sucrose will retard slightly the development of oxidized flavor.

It has been shown (11) that nordihydroguaiaretic acid (NDGA) will retard the development of oxidized flavor in unsweetened frozen cream. The work reported herein consists of a study in which NDGA was used to retard the development of oxidized flavor during the storage of sweetened frozen cream.

EXPERIMENTAL PROCEDURE

Cream of both high and low quality was used in this study. Standard plate count (1), acidity and pH were the quality criteria (table 1).

TABLE 1

The standard plate count, titratable acidity and pH of the raw and pasteurized cream

	Low quality		High quality	
<i>Raw Cream</i>	<i>Series A & B</i>	<i>Series E & F</i>	<i>Series C & D</i>	<i>Series G & H</i>
Standard plate count/ml.	1,000,000	600,000	135,000	180,000
Titratable acidity (as % lactic acid)	0.19	0.18	0.125	0.13
pH (25° C.)	6.70	6.70	6.90	6.80
<i>Pasteurized Cream</i>	<i>150° F.</i>	<i>170° F.</i>	<i>150° F.</i>	<i>170° F.</i>
Standard plate count/ml.	5,500	500	300	400
Titratable acidity (as % lactic acid)	0.19	0.17	0.13	0.13
pH (25° C.)	6.65	6.70	6.75	6.90

The different batches of cream were standardized to contain 40 per cent milk fat and 10 per cent sucrose. NDGA was added before pasteurization as a 15 per cent solution in propylene glycol or as a 5 per cent water suspension. The concentrations of NDGA were computed on the basis of the fat content of the cream. When used, copper was added before pasteurization at a concentration of 0.5 p.p.m. as a 0.5 per cent aqueous solution of copper sulfate.

The cream was pasteurized in well-tinned equipment, cooled to 40 to 45° F., sealed in tinned cans holding 300 ml. and stored at -12 to -20° F. To examine for monthly flavor defects the frozen cream was thawed by holding it 24 hours at 40° F. It was then held at 40° F. for 1 week and again examined for flavor defects. The judging panel consisted of three or more persons.

Received for publication October 26, 1948.

RESULTS

The data in table 2 are typical of results obtained with cream placed in storage during January and February. There were no significant differences in the antioxygenic effectiveness of the NDGA when added in propylene glycol solution and when added in a water suspension. Therefore, the data presented in table 2 include only results from cream treated with NDGA in propylene glycol solution.

The effect of concentration of NDGA. A concentration of 0.005 per cent of the antioxidant was more effective than one of 0.00125 per cent. This is shown in the results obtained with the cream of series D (table 2) at the end of 12 months and the cream of series B after storage for 8, 10 and 12 months. In the former case, oxidized flavor was present at the end of storage for 12 months at sub-zero temperatures plus 1 week at 40° F. in the cream containing 0.00125 per cent NDGA, but was not present in the cream which contained 0.005 per cent NDGA. In the latter case, oxidized flavor was present at the end of storage for 8 months at sub-zero temperatures in the cream containing 0.00125 per cent NDGA but was not detected in the cream containing 0.005 per cent antioxidant until the end of 12 months; at that time the intensity of the off flavor was less in the sample containing 0.005 per cent NDGA.

The effect of pasteurization temperature. Oxidized flavor development was retarded by pasteurization at 170° F. for 15 minutes. This substantiates the results obtained in other investigations dealing with the factors affecting the keeping quality of frozen cream (2, 5, 11).

Oxidized flavor was present after 2 months in the control sample of series B while it did not develop during storage at sub-zero temperatures until after 10 months in the similar cream (series F) which was pasteurized at 170° F. for 15 minutes. There was no oxidized flavor in the cream of series G, while the off-flavor was present at the end of 8 months storage in the control sample of the cream (series C) pasteurized at 150° F. for 30 minutes. The oxidized flavor was present after storage for 2 months in the control sample of series D while there was no development of typical oxidized flavor in the control sample of the similar cream (series H) pasteurized at 170° F. for 15 minutes.

While keeping quality of the control samples of the cream pasteurized at 170° F. for 15 minutes was superior to those pasteurized at 150° F. for 30 minutes, the former had a cooked flavor which persisted throughout the storage period. The keeping quality of the cream which was pasteurized at 150° F. for 30 minutes and which contained NDGA was comparable to that of the cream pasteurized at 170° F. for 15 minutes but which contained no added antioxidant.

The effect of quality of the cream. Oxidized flavor was present at the end of 8 months storage in the control sample of the high-quality cream of series C which contained no added copper, whereas there was no oxidized flavor development in the similar low-quality cream (series A). There was a similar difference in the keeping quality of the low- and high-quality cream containing added copper (series B and D, respectively).

TABLE 2
The antioxidant effect of NDGA added to sweetened cream stored at sub-zero temperatures

Treatment	Flavor criticisms											
	2 mo.		4 mo.		6 mo.		8 mo.		10 mo.		12 mo.	
	(1) ^a	(2) ^b	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
<i>Pasteurized at 160° F. for 30 min.</i>												
Series A. Low quality cream. No copper added. No oxidized flavor in control, control + 0.00125% NDGA and control + 0.005% NDGA												
Control												
Control + 0.00125% NDGA	1		3		1	3	2	3	2	3	3	5
Control + 0.005% NDGA	- ^d	-	-	-	-	-	-	-	-	-	-	1
Series B. Low quality cream. 0.5 p.p.m. copper added.												
Control	1											
Control + 0.00125% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Control + 0.005% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Series C. High quality cream. No copper added.												
Control	1		2		± ^e	3	1	4	3	4	3	4
Control + 0.00125% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Control + 0.005% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Series D. High quality cream. 0.5 p.p.m. copper added.												
Control	1	2	3		3	4	4	5	4	5	4	5
Control + 0.00125% NDGA	-	-	-	-	-	-	1	1	1	1	2	2
Control + 0.005% NDGA	-	-	-	-	-	-	-	-	-	-	1	1
<i>Pasteurized at 170° F. for 15 min.</i>												
Series E. Low quality cream. No copper added. No oxidized flavor in control, control + 0.00125% NDGA and control + 0.005% NDGA												
Control												
Control + 0.00125% NDGA	-	±	1		±	1	±	1	1	2	2	2
Control + 0.005% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Series F. Low quality cream. 0.5 p.p.m. copper added.												
Control	-	-	±		±	1	±	1	1	2	2	2
Control + 0.00125% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Control + 0.005% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Series G. Low quality cream. No copper added. No oxidized flavor in control, control + 0.00125% NDGA and control + 0.005% NDGA												
Control												
Control + 0.00125% NDGA	-	-	±		-	±	±	±	±	±	±	±
Control + 0.005% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Series H. High quality cream. 0.5 p.p.m. copper added.												
Control	-	±	±		-	±	±	±	±	±	±	±
Control + 0.00125% NDGA	-	-	-	-	-	-	-	-	-	-	-	-
Control + 0.005% NDGA	-	-	-	-	-	-	-	-	-	-	-	-

^a Flavor judged immediately after taking cream out of storage and thawing it.

^b Flavor judged after thawing cream and then holding it at 40° F. for 1 week.

^c The numbers 1 to 5 indicate increasing intensities of the oxidized flavor defect.

^d No oxidized flavor present.

^e Flavor slightly 'off'. Not typically oxidized.

In the case of cream pasteurized at 170° F. for 15 minutes, there was little difference in the keeping quality of the high- and low-quality cream. The control sample of the low quality cream (series F) developed oxidized flavor at the end of storage for 10 months but the similar high quality cream (series H) did not develop the typical oxidized flavor during 12 months storage.

In the study, therefore, the low quality cream which was pasteurized at 150° F. for 30 minutes had a better keeping quality than the high quality cream pasteurized in the same manner. In the case of cream pasteurized at 170° F. for 15 minutes, the high quality cream had better keeping quality than the low quality cream pasteurized in the same manner.

The effect of holding the thawed cream at 40° F. for 1 week. Oxidized flavor sometimes developed in the cream which was held at 40° F. for 1 week although it did not have that off-flavor when it first was taken out of storage at sub-zero temperatures. This relationship was illustrated in the sample containing 0.00125 per cent NDGA in series B at the end of 12 months, in the control sample of series C at the end of 2, 4 and 6 months, and in the control sample of series F at the end of 4, 6 and 8 months.

After storage for 1 week at 40° F., oxidized flavor usually increased in intensity in the control samples which had that off-flavor when they first were taken out of storage. This is the case in the control samples of series B at the end of 4, 6, 8, 10 and 12 months, series C at the end of 8, 10 and 12 months, series D at the end of 2 months and Series F at the end of 10 and 12 months. However, the intensity of oxidized flavor did not increase during storage at 40° F. in the samples which contained NDGA. This is shown in series D at the end of 8, 10 and 12 months in the cream containing 0.00125 per cent NDGA and at the end of 12 months in the cream containing 0.005 per cent NDGA.

CONCLUSIONS

1. Concentrations of 0.00125 to 0.005 per cent (butterfat weight basis) nordihydroguaiaretic acid were found to retard the development of oxidized flavor in sweetened frozen cream during storage for 12 months.

2. In the absence of added copper, the keeping quality of the cream which contained nordihydroguaiaretic acid and was pasteurized at 150° F. for 30 minutes was comparable to that pasteurized at 170° F. for 15 minutes but to which the antioxidant had not been added.

3. In this study, the low quality cream pasteurized at 150° F. for 30 minutes had a better keeping quality than the high quality cream similarly pasteurized.

4. During storage for 1 week at 40° F., oxidized flavor developed frequently in the control samples which did not have the off-flavor when they first were taken out of storage at sub-zero temperatures. This development of oxidized flavor occurred only in one instance in the cream which contained nordihydroguaiaretic acid.

5. During storage for 1 week at 40° F., the intensity of oxidized flavor usually increased in the control samples which had the off-flavor when they

first were taken out of storage at sub-zero temperatures. This did not occur in the oxidized samples which contained nordihydroguaiaretic acid.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 9th ed. Am. Pub. Health Assoc., New York. Pp. 85-107. 1948.
- (2) DAHLE, C. D., LAWHORN, R. K., AND BARNHART, J. L. Factors Affecting the Keeping Quality of Frozen Cream. Proc. Intern. Assoc. Ice Cream Mfrs., 2: 7-23. 1940.
- (3) DOAN, F. J., AND BALDWIN, F. B., JR. Observations on the Freezing of Milk and Cream. II. Destruction of the Fat Emulsion in Frozen Cream. J. Dairy Sci., 19: 225-235. 1936.
- (4) JOSEPHSON, D. V., AND DAHLE, C. D. The Importance of the Fat Globule Membrane in the Freezing of Ice Cream. Rept. Proc. Intern. Assoc. Ice Cream Mfrs. 36th Ann. Conv., 1936, 2: 100-112. 1936.
- (5) MCFARLAND, G. C., AND BURGWARD, L. H. Prevention of Oxidized Flavor in Frozen Cream by Homogenization and High Temperature Pasteurization. J. Dairy Sci., 23: 494-495. 1940.
- (6) MACK, M. J. Frozen Cream as an Ingredient of Ice Cream. Mass. Agr. Expt. Sta. Bull. 268. 1930.
- (7) MACK, M. J. Use of Frozen Sweet Cream as an Ice Cream Ingredient. Ice Cream Trade J., 26: 50-53. 1930.
- (8) PRICE, W. V. The Influence of Sweetened Frozen Cream on the Development of Swell in Ice Cream. J. Dairy Sci., 14: 221-229. 1931.
- (9) PRICE, W. V. Sugar Improves Keeping Quality of Cream. Wis. Agr. Expt. Sta. Bull. 421. 1932.
- (10) PRICE, W. V. Use of Frozen Cream in Ice Cream. Am. Creamery and Poultry Prod. Rev., 74: 210-217. 1932.
- (11) STULL, J. W., HERREID, E. O., AND TRACY, P. H. A Study of the Use of Nordihydroguaiaretic Acid in Dairy Products. II. Its Antioxygenic Properties in Unsweetened Frozen Cream. J. Dairy Sci., 31: 1024-1029. 1948.
- (12) TROUT, G. M., AND SCHEID, M. V. The Influence of Several Factors Upon the Flavor of Frozen Sweet Cream. J. Dairy Sci., 26: 609-619. 1943.
- (13) WEBB, B. H., AND HALL, S. A. Some Physical Effects of Freezing Upon Milk and Cream. J. Dairy Sci., 18: 275-287. 1935.

THE RELATIONSHIP OF ASCORBIC ACID TO THE DEVELOPMENT OF OXIDIZED FLAVOR IN MARKET MILK

W. H. CHILSON, W. H. MARTIN, AND D. B. PARRISH

Kansas Agricultural Experiment Station

The flavor known as oxidized and often called cappy, cardboard, oily or tallowy, is one of the most troublesome off flavors in milk, especially during the winter months. Although much work has been done on oxidized flavor during the past quarter century, neither the cause nor the remedy of the defect has been determined satisfactorily. Data recently published seem somewhat contradictory to previous work on oxidized flavor. This study was conducted to gain more knowledge of the causative factors involved in the development of the oxidized flavor of milk and to seek some practical control measures.

REVIEW OF LITERATURE

This review will include only the work which seems to have a direct bearing upon this particular study.

After reviewing past research on the development of oxidized flavor in milk, Brown and Thurston (1) concluded that copper, sunlight and the presence of oxygen promote the development of oxidized flavor. Milk known as "spontaneous" milk will develop an oxidized flavor without the aid of either copper or light. Ascorbic acid and other anti-oxidants were found to retard or inhibit the development of oxidized flavor when added to milk. Their review of some research indicates several other factors such as feed, temperature and season that may be involved in the production or inhibition of oxidized flavor under commercial conditions. A more recent review by Greenbank (6) summarizes the early work and emphasizes newer research and theories. The possible role of ascorbic acid in the development and inhibition of oxidized flavor is well presented in this review.

Beck *et al.* (2) found a marked relationship between color intensity or carotene content of milk and its susceptibility to oxidized flavor, the higher carotene milk being less susceptible. They were successful in preventing oxidized flavor in raw milk by feeding a concentrate containing as little as 206 mg. of carotene per head daily to cows that consistently had been producing milk with this off flavor.

Josephson *et al.* (7) found that under experimental conditions of exposing milk to sunlight, the loss of ascorbic acid was rapid, with only insignificant quantities remaining after 30 minutes. Even in the shade the loss of this vitamin was quite rapid. In their survey of route deliveries they found that 70 percent of all retail milk is removed from the doorstep within 5 minutes after delivery, or is protected from light. The loss of ascorbic acid was found to

Received for publication October 27, 1948.

Contribution no. 179, Department of Dairy Husbandry, and no. 375, Department of Chemistry.

be considerable while the milk was in transit if not properly protected from direct light.

Chilson (3) found that by adding ascorbic acid to pasteurized milk processed under laboratory conditions the development of the oxidized flavor could be retarded for several days. He suggested that an enzyme was present in skim milk which catalyzed the oxidation of the fat, since the oxidized flavor did not develop in milk prepared from skim milk which had been heated to 170° F. for 30 minutes and then mixed with unheated cream. He also reported no appreciable loss of ascorbic acid when milk was heated to 200° F. and held for 30 minutes, but about 7 per cent loss occurred when milk was held at 143° F. for 30 minutes. It was assumed that the heating of milk to above 170° F. destroyed the oxidizing enzyme which was responsible for the destruction of ascorbic acid and the development of this off flavor.

Gjessing and Trout (4) found that milk pasteurized at 67° C. (167° F.) for 30 minutes exhibited marked stability of ascorbic acid with no development of oxidized flavor after storage for 3 days, even when 0.13 mg. of copper per l. had been added. They believed that the protection was due to the liberation of sulphides and other reducing agents at this high temperature.

Olson and Brown (9) have demonstrated that washed cream from susceptible milk does not develop an oxidized flavor when contaminated with copper and stored for 3 days. However, when washed cream from the same source, likewise contaminated with copper, was fortified with ascorbic acid, a strong oxidized flavor developed.

More recent work by Krukovsky and Guthrie (8) has shown that complete and rapid destruction of ascorbic acid by light or hydrogen peroxide will prevent the development of an oxidized flavor. These workers also have shown that ascorbic acid is an important link in a chain reaction promoting oxidation of milk fat.

Greenbank (5) explains the effect of either addition or destruction of ascorbic acid in relation to changes in the oxidation-reduction potential. The ability of a sample of milk to develop an oxidized flavor seems to be related closely to the Eh value and the poisoning action of the milk. However, some workers do not consider the O-R potential as a reliable index to oxidized flavor development (10).

METHODS

In preliminary studies prior to the organization of this experiment, six lots of milk collected during November and December were treated with 1.5 g. of ascorbic acid per 100 lb. of milk. These samples were stored for 5 days and examined for oxidized flavor after 0, 2, 3 and 5 days. The six control samples developed a distinct oxidized flavor by the fifth day or earlier, while no oxidized flavor was detectable in any samples treated with ascorbic acid. In these comparisons no chemical analyses were made to determine the amounts of ascorbic acid present or the rate of destruction during storage.

In the first part of this investigation the relationship between the destruc-

tion of ascorbic acid in milk and the incidence of oxidized flavor was studied. The two trials, each consisting of four split batches of milk, represented a comparison between milk fortified with ascorbic acid and non-fortified milk. The first trial was conducted in February and March when the cows were on winter feed and the second trial in April and May when the cows were on grass.

In the second part of this investigation the effect of subjecting the milk to various light exposures upon the ascorbic acid content and the incidence of oxidized flavors was studied. All milk used was fortified with ascorbic acid as described above. In three trials the ascorbic acid content was determined before and after exposure to light, and each sample was analyzed for ascorbic acid and tasted for oxidized flavor daily during the 5-day storage period.

In the third part of the investigation the incidence of oxidized flavor was compared when samples were treated by adding 30 mg. of ascorbic acid per l. of milk to inhibit development of the flavor, and when the ascorbic acid was destroyed by exposure of the milk to intense sunlight or by oxidation resulting from the addition of 0.1 ml. of 30 per cent hydrogen peroxide per l. of milk. The effect of sunlight upon flavor also was tested by subjecting to sunlight the milk in which the ascorbic acid had been destroyed through the addition of hydrogen peroxide. The exposure to direct sunlight was for 0.5 hour in each instance.

In the fourth part of this investigation a preliminary test was made, whereby samples of milk were subjected to conditions similar to those of commercial handling and retail delivery. In the first trial, milk fortified with ascorbic acid was stored 24 hours, then carried in a covered delivery truck in standard bottle cases for 3 hours (5 to 8 a.m.) and then exposed to daylight in the shade for 0.5 hour (8 to 8:30 a.m.). Ascorbic acid determinations were made after each successive treatment and the milk was examined for oxidized flavor after storage for 3, 5 and 7 days. In the second part of the trial three quarts of unfortified milk were taken from the bottle filler; one quart was placed in storage as a control, and two quarts were carried on the delivery truck from 9:30 a.m. to 12 noon. Then one bottle was placed in the shade for 1 hour while the other bottle was subjected to direct sunlight for 15 minutes.

The milk used for comparisons was pasteurized by the holder method. All equipment used was of stainless steel construction except the cooler which was tinned copper with no copper exposed. Samples were stored for from 0 to 7 days in a cabinet refrigerator at 35 to 40° F. and protected from light. The ascorbic acid content of the original milk and the milk at selected periods during storage was determined by the method of Woessner *et al.* (11). All samples were tested organoleptically by two experienced judges of milk flavors at the same period when analyzed for ascorbic acid content. The ascorbic acid was added to the milk in the vat after pasteurization previous to cooling, at the rate of 1.5 g. per 100 lb. of milk. Ascorbic acid powder, sufficient for 100 gallons of milk, was dissolved in a pint of water before being added to the milk. The milk used was from the Kansas State College herd and had been found susceptible to the development of oxidized flavor.

RESULTS

Both the control milk and the ascorbic acid fortified milk averaged slightly higher in ascorbic acid content when the cows were grass-fed than when cows were fed winter rations (table 1). However, the addition of 1.5 g. of ascorbic acid per 100 lb. of milk increased the ascorbic acid content of the fortified milk to approximately three times that of the control milk in both trials. The average losses of ascorbic acid in the two trials with control milk were quite similar; they were in excess of one-fifth of the original content after one day's storage, about one-half by the second day, three-fourths by the third day, and more than 90 per cent after 5 days' storage. Although more milligrams of ascorbic acid were lost daily in the fortified milk than in the non-fortified, in general, the percentage loss was at a much less rapid rate. The average losses with fortified milk were quite similar in both trials; the loss during the first day of storage was less than one-tenth in both trials, slightly more than one-fifth for both trials after 2 days, about one-third by the third day, and about one-half of the original after 5 days of storage. Even after 5 days of storage, the fortified milk contained about one-half more ascorbic acid than the control milk contained originally. No oxidized flavors were detected in the fortified milk during a 5-day storage period in the first trial or during a 7-day storage in the second trial. However, in the control samples, oxidized flavors began to develop on the second day of storage and became increasingly frequent with longer storage periods. These results indicate that the addition of 1.5 g. of ascorbic acid to 100 lb. of milk was sufficient to inhibit the development of oxidized flavor during a normal storage period. The general effect of grass feeding was to delay the appearance of oxidized flavor by 1 additional day. This could be of considerable importance in commercial milk distribution.

Results of three trials to determine the effects of various periods of exposure and various intensities of light upon the ascorbic acid content of fortified milk are shown in table 2. In trial I, exposure of milk for 1 hour in the shade caused an immediate reduction from 38.3 to 23.2 mg. (about 40 per cent) in the ascorbic acid content, while exposure for the same period to direct sunlight caused a reduction from 38.3 to 8.6 mg. (about 78 per cent). Further reductions in ascorbic acid occurred throughout the period of storage. Oxidized flavors were detected on the third day of storage in milk that had been exposed to daylight in shade, but milk exposed to direct sunlight had a pronounced oxidized flavor after only 1 day of storage.

In trial II exposure of milk to daylight on a cloudy day for one-half hour caused a loss from 43.6 to 27.9 mg. (about 36 per cent) of the ascorbic acid present, and oxidized flavor developed after only 1 day of storage. The instability of ascorbic acid when milk is exposed to direct sunlight was measured in more detail during trial III. Milk exposed for 5 minutes lost approximately half of its ascorbic acid, and milk exposed 20 minutes lost about 90 per cent, while milk exposed for 40 minutes lost practically all ascorbic acid. The oxidized flavor was detected after storage for 1 day of milk which had been ex-

TABLE 1
The relationship of ascorbic acid destruction and the development of oxidized flavor in pasteurized milk

Trial	Number of Samples	Treatment	Days Stored							Days Stored						
			0	1	2	3	5	7		1	2	3	5	7		
I Feb. March (Winter ration)	4	None	av. mg. ascorbic acid/l. milk and % loss							frequency of occurrence and intensity of oxidized flavor						
			14.7	11.6	7.4	3.4	1.3			4-a b	2++	2++	1+++			
		21%	50%	77%	91%				2-	2-	2+++	3+++				
		4	48.6	44.0	37.5	33.2	24.3			4-	4-	4-	4-			
II April May (Grass-fed)	4	None	1.5 g. ascorbic acid added/100 lb. milk													
			9%	23%	32%	50%										
		4	17.2	12.8	8.1	4.4	1.1	1.1		4-	4-	1+	1+	2++		
			26%	26%	53%	74%	94%	94%			3-	3++	2+++			
	4	1.5 g. ascorbic acid added/100 lb. milk														
			51.2	48.6	40.6	34.3	26.4	18.6		4-	4-	4-	4-	4-		
		4	5%	21%	33%	48%	64%									

a Figure indicates number of samples in each classification.

b - indicates no oxidized flavor.

c +, ++, +++ indicate increasing intensity of oxidized flavor.

TABLE 2
The relationship of time milk is exposed to light, and the ascorbic acid content and the development of oxidized flavor

Trial	Sample and treatment ^a	Ascorbic acid content	Days Stored				
			1	2	3	4	5
I ^b	Not exposed ^c	38.3	mg. of ascorbic acid/l. milk and oxidized flavor value				
	Exposed 1 hr. to daylight in shade	23.2	18.7	18.3	+++	+++	+++
	Exposed 1 hr. to direct sunlight	8.6	6.4	6.0	+++	+++	+++
II ^b	Not exposed	43.6	37.7	32.0			
	Exposed 0.5 hr. to daylight	27.9	23.1	18.5	++		
III ^b ^d	Not exposed	43.7	-	-	-	-	-
	Exposed 5 min. to direct sunlight	22.6	+	+++	++	++	++
	" 10 min. " " "	12.2	++	+++	+++	+++	+++
	" 20 min. " " "	4.6	++	++	+++	+++	+++
	" 40 min. " " "	1.2	-	-	-	-	-
	" 60 min. " " "	1.2	-	-	-	-	-

^a All milk used in these trials was fortified with approximately 1.5 g. of ascorbic acid per 100 lb. of milk.

^b Trial I, clear day (9-10 a.m., Mar. 8, 1948); trial II, cloudy day (9:30-10 a.m., Mar. 10, 1948); trial III, clear day (10:50-11:50 a.m., Mar. 19, 1948).

^c Stored samples not checked for flavor.

^d Ascorbic acid content of stored samples not determined.

- indicates no oxidized flavor.

+, ++, +++ indicate increasing intensity of oxidized flavor.

posed 5, 10 or 20 minutes. On succeeding days of storage the oxidized flavor became more pronounced. No oxidized flavor was detected in any of the stored samples of milk which had been exposed 40 or 60 minutes to direct sunlight. It would seem that the rapid and rather complete reduction in the ascorbic acid content of the milk tended to inhibit the development of the oxidized flavor. These latter samples had a pronounced cooked or burnt flavor after 24 hours of storage, but the off flavor gradually disappeared as storage time was extended.

Krukovsky and Guthrie (8) have reported that oxidized flavor in milk can be inhibited by the complete destruction of ascorbic acid through either exposure of the milk to direct sunlight or the addition of hydrogen peroxide to the milk to oxidize the ascorbic acid. A preliminary trial was conducted to compare ascorbic acid-fortified milk with milk having all the ascorbic acid removed by the use of either hydrogen peroxide or sunlight (table 3). The

TABLE 3

The relationship of ascorbic acid, hydrogen peroxide, and sunlight to the development of the oxidized flavor

Sample	Ascorbic acid/l. of milk	Days stored				
		1	2	3	5	
		Oxidized flavor value				
		(mg.)				
1. Control milk		- ^a	-	+	++	
2. No. 1 + 30 mg. ascorbic acid/l.		-	-	-	-	
3. No. 1 + 0.1 ml. 30% H ₂ O ₂ /l.	1.1	-	-	-	-	
4. No. 2 + 0.1 ml. 30% H ₂ O ₂ /l.	1.6	-	-	-	-	
5. No. 1 exposed 1.5 hr. to direct sunlight	0.6	-	-	-	-	
6. No. 1 exposed 0.5 hr. to direct sunlight		-	+	+	++	
7. No. 2 exposed 0.5 hr. to direct sunlight		+++ ^b	++++	++++	++++	
8. No. 3 exposed 0.5 hr. to direct sunlight		-	-	-	-	
9. No. 4 exposed 0.5 hr. to direct sunlight		-	-	-	-	

^a - indicates no oxidized flavor.

^b +, ++, +++, ++++ indicate increasing intensity of oxidized flavor.

stored control sample developed an oxidized flavor in 3 to 5 days, indicating the susceptibility of the milk. The addition of ascorbic acid to the control milk inhibited the development of the off flavor during the 5-day observation period. The addition of 0.1 ml. of 30 per cent hydrogen peroxide to 1 l. of milk removed essentially all quantities of ascorbic acid from both the control milk and the fortified milk. Neither milk showed evidence of oxidized flavor.

Exposure of a sample of the milk to direct sunlight for 1.5 hours also removed essentially all ascorbic acid. No oxidized flavor developed in this milk during 5 days of storage. However, when a sample was exposed to direct sunlight for 0.5 hour, an oxidized flavor developed after 2 days of storage. Exposure of the fortified milk to direct sunlight for 0.5 hour caused the development of a pronounced oxidized flavor after 1 day in storage. When the samples to which hydrogen peroxide had been added were exposed to 0.5 hour of direct

sunlight, no oxidized flavor resulted during 5 days of storage. This indicates that ascorbic acid must be present before the sunlight can cause the development of the oxidized flavor. Other samples were treated with hydrogen peroxide and gave results similar to those shown in table 3.

The results of a study on milk subjected to commercial handling conditions were as follows: Fortified milk containing 46 mg. of ascorbic acid per l. lost about one-sixth of the ascorbic acid after 24 hours storage, only a slight additional amount was lost while on the truck and about one-third of the remaining ascorbic acid was lost when exposed 0.5 hour in the shade. Unfortified milk containing 13.2 mg. of ascorbic acid per l. showed no loss of ascorbic acid after 2.5 hours on the truck, but about one-half was lost when exposed for 1 hour in the shade; practically all the ascorbic acid was destroyed after 15 minutes of exposure to direct sunlight. No oxidized flavor developed in any of these samples, even after 7 days storage, showing that the milk at this time was much less susceptible to oxidation than the milk used in the previous experiments.

DISCUSSION

From a review of the literature and the results of these experiments, it appears that oxidized flavor in susceptible milk can be controlled either by the addition of ascorbic acid to the milk or by the complete and rapid oxidation of all ascorbic acid present in the milk. When ascorbic acid is added to milk it acts as a reducing agent which oxidizes more readily than the milk fat, therefore either preventing or prolonging the time required for fat oxidation and the development of an oxidized flavor (3). When the ascorbic acid is oxidized completely and rapidly, the development of oxidized flavor is prevented (8). Theories on the chemical reactions involved in this procedure are somewhat controversial. At present, the addition of ascorbic acid is the only method of commercial interest. It now has been established that the oxidized flavor can be controlled for several days by the addition of ascorbic acid if the milk supply is free from copper and is protected from sunlight. The price of ascorbic acid is low enough to justify its use as a flavor control measure when necessary. Ascorbic acid is tasteless in the milk and, being a natural food substance, any excess at the time of consumption will add to the food value of the milk. Ascorbic acid also is relatively easy to add, no expensive equipment being necessary. However, its use presents a number of problems when considered for commercial use, such as approval by regulatory authorities, proper handling of the milk in relation to sunlight and possible deceptive labeling to show increased vitamin C content of the milk. Due to the instability of ascorbic acid under many conditions, no guarantee should be made regarding the vitamin C content of milk at the time of either delivery or consumption. The protection of the milk from sunlight during the transportation period to the customer's refrigerator would be the greatest problem for many dairies. Present knowledge would indicate only wasted effort if ascorbic acid were added to milk not protected from sunlight at all times. Under certain conditions, a

more objectionable oxidized flavor might be developed in milk with added ascorbic acid than would be encountered in the original milk (table 3).

It has been established that oxidized flavor can be controlled by the rapid and complete removal of ascorbic acid by several methods (8). (The measurement of ascorbic acid may be used as a guide to the inhibition of flavor development. However, it is not established fully that the oxidation of ascorbic acid is the key factor to the development or inhibition of the oxidized flavor.) There are no commercial methods now available for the rapid destruction of ascorbic acid in large quantities of milk. Should machinery be developed which will remove the ascorbic acid economically and rapidly in the line of processing, this method may hold the greatest possibility for future control of oxidized flavors. The greatest disadvantage of the removal of ascorbic acid would be the difficulty in obtaining the approval of a process by the public and the medical profession. Willful removal of a natural vitamin from a universally-used food may be questioned. However, if a survey were made of all milk at the time of consumption, there is little doubt that most of it would have insignificant quantities of vitamin C present.

SUMMARY

The addition of ascorbic acid to milk at the rate of 1.5 g. to 100 lb. resulted in a three-fold increase in ascorbic acid content of the milk. Non-fortified milk lost more than one-fifth of its ascorbic acid during the first day of refrigerated storage in the dark, about half by the second day, three-fourths by the third day, and better than 90 per cent by the fifth day. Milk fortified with ascorbic acid lost less than one-tenth during the first day, slightly more than one-fifth the second day, about one-third by the third day, and about one-half after 5 days of storage. After 5 days the fortified samples contained about one-half more ascorbic acid than the control milk did originally. No oxidized flavors developed in the fortified milk in 5- or 7-day storage periods, while the control samples began to develop oxidized flavors after 2 days of storage and became increasingly worse as storage time extended.

Milk fortified with ascorbic acid rapidly lost its ascorbic acid when exposed to light, the rate depending upon the intensity of light and the time exposed. When exposed to direct sunlight for 40 minutes, all measurable ascorbic acid was destroyed. Stored samples in which ascorbic acid was destroyed partially developed an oxidized flavor.

Rapid destruction of all measurable ascorbic acid in milk either by exposure to direct sunlight or through the addition of 30 per cent hydrogen peroxide resulted in no oxidized flavors over a 5-day storage period, indicating that ascorbic acid is a contributing factor in the development of this off flavor. Milk in which the ascorbic acid was destroyed by the addition of hydrogen peroxide did not develop oxidized flavor when exposed to sunlight.

A study on the destruction of ascorbic acid and the development of oxidized flavor in milk under commercial conditions showed that about one-sixth of the ascorbic acid was lost from a fortified sample (30 mg. per l.) after 24

hours of storage. The same milk lost only a slight amount of ascorbic acid when carried in a case in a covered milk truck from 5 to 8 a.m. When this milk then was exposed to daylight in the shade for 30 minutes it lost about one-third of the remaining ascorbic acid. Another trial with non-fortified milk showed no loss of ascorbic acid while carried in a case on a covered milk truck for 2.5 hours. About one-half of the ascorbic acid was lost, however, when this milk was exposed to daylight in the shade for 1 hour at noontime. Exposure of the milk to direct sunlight for 15 minutes at noon destroyed all measurable quantities of the ascorbic acid. These results indicate that most of the destruction of ascorbic acid occurs after the milk leaves the truck.

ACKNOWLEDGMENTS

Roy Coleman assisted with some of the technical work in chemistry. The ascorbic acid used in these experiments was furnished by Hoffman-La Roche, Inc., Nutley, New Jersey.

REFERENCES

- (1) BROWN, W. CARSON, AND THURSTON, L. M. A Review of Oxidation in Milk and Milk Products as Related to Flavor. *J. Dairy Sci.*, **23**: 629-685. 1940.
- (2) BECK, G. H., WHITNAH, C. H., AND MARTIN, W. H. Relation of Vitamin C, Lecithin, and Carotene of Milk to the Development of Oxidized Flavor. *J. Dairy Sci.*, **22**: 17-29. 1939.
- (3) CHILSON, W. H. What Causes Most Common Off Flavors of Market Milk. *Milk Plant Monthly*, **24**, 11: 24-28 and **24**, 12: 30-34. 1935.
- (4) GJESSING, E. C., AND TROUT, G. M. Ascorbic Acid and Oxidized Flavor in Milk. II. The Effect of Various Heat Treatments of Milk upon the Stability of Ascorbic Acid and upon the Development of Oxidized Flavor. *J. Dairy Sci.*, **23**: 373-384. 1940.
- (5) GREENBANK, GEORGE R. Variation in the Oxidation-Reduction Potential as a Cause for the Oxidized Flavor in Milk. *J. Dairy Sci.*, **23**: 725-744. 1940.
- (6) GREENBANK, GEORGE R. The Oxidized Flavor in Milk and Dairy Products: A Review. *J. Dairy Sci.*, **31**: 913-933. 1948.
- (7) JOSEPHSON, D. V., RUBGWALD, L. H., AND STOLTZ, R. B. The Effect of Route Delivery on the Flavor, Riboflavin and Ascorbic Acid Content of Milk. *J. Dairy Sci.*, **29**: 273-284. 1946.
- (8) KEUKOVSKY, V. N., AND GUTHRIE, E. S. Ascorbic Acid Oxidation a Key Factor in the Inhibition or Promotion of the Tallowy Flavor in Milk. *J. Dairy Sci.*, **28**: 565-579. 1945.
- (9) OLSON, FLOYD C., AND BROWN, W. CARSON. Oxidized Flavor in Milk XI. *J. Dairy Sci.*, **25**: 1023-1027. 1942.
- (10) SWANSON, A. M., AND SOMMER, H. H. Oxidized Flavor in Milk. II. The Relation of Oxidation-Reduction Potentials to its Development. *J. Dairy Sci.*, **23**: 597-614. 1940.
- (11) WOESSNER, W. W., ELVEHEJEM, C. A., AND SCHUETTE, H. R. The Determination of Ascorbic Acid in Commercial Milks. *J. Nutrition*, **18**: 619. 1939.

THE INFLUENCE OF TEMPERATURE OF RIPENING ON THE TYRAMINE CONTENT AND FLAVOR OF AMERICAN CHEDDAR CHEESE¹

A. C. DAHLBERG AND F. V. KOSIKOWSKY

Department of Dairy Industry, Cornell University, Ithaca, New York

It has been shown recently that a special strain of *Streptococcus faecalis*, which produced acid rapidly in milk and which grew in cheese, increased the rate of ripening and the quality of American Cheddar cheese (2, 5). This culture produced tyramine in cheese, and the amount of tyramine in both commercial and experimental cheese was directly related to the intensity of Cheddar flavor (6, 3). Within the range of usual temperatures for ripening cheese, it is known that ripening is accelerated as the temperature increases. It was the purpose of the present study to determine the influence of ripening temperatures upon the rate of production of tyramine in American Cheddar cheese made with and without *S. faecalis* starter and the usual commercial lactic starter.

EXPERIMENTAL METHODS

The Cheddar cheese was made from pasteurized milk. One batch was made on March 20, 1947, from milk produced by the herds of Cornell University, and another on April 3, 1947, from milk produced for sale as fluid milk for New York City. After mixing in the vat pasteurizer, each batch was divided into 3 parts. To one portion 2 per cent of Hansen's lactic starter was added, to another portion 2 per cent of *S. faecalis* starter was added, and to the third portion 1 per cent of lactic starter and 1 per cent of *S. faecalis* starter were added. The milk was made into cheese without giving time for the starter to develop before adding rennet, but otherwise following the time schedule of Wilson (7). All lots made up quite uniformly so the manufacturing data are not presented. The cheese was packed in tin cans containing 1 lb. of cheese sealed under 25 inches of vacuum, and the cans showed 20 to 22 inches of vacuum after sealing.

Each batch of cheese was divided into 3 lots for ripening at 40, 50 and 60° F. The cheese was placed in the curing rooms the day it was taken from the press. Samples of the cheese were analyzed for tyramine at intervals up to 1 year by the method of Kosikowsky and Dahlberg (6), for volatile acids by the method of Kosikowsky and Dahlberg (4), and for water soluble protein by the method of Sharp as reported by Dahlberg and Kosikowsky (1). The scoring was done by the authors and was discontinued after 6 months as the cheese held at 60° F. was definitely overcured in this period of time.

RESULTS

The composition of the three lots of fresh cheese was reasonably uniform and well within the range of acceptable Cheddar cheese (table 1). The fresh

Received for publication November 4, 1948.

¹ This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Catherine Verwoert Work for making some of the chemical analyses.

TABLE 1
Composition of the fresh cheese

	Starter used in making the cheese		
	Lactic	<i>faecalis</i>	Lactic <i>faecalis</i>
Moisture (%)	36.07	37.74	36.16
Fat, (%)	35.0	33.5	34.5
Protein, (%)	23.61	23.65	23.40
Salt, (%)	1.62	1.55	1.67
pH	5.08	5.29	5.23

cheese made with *S. faecalis* starter only was slightly less acid and slightly higher in moisture, as *S. faecalis* starter produces acid about half as rapidly as commercial lactic starter and no correction was made for this difference by variations in the amount of starter added to the milk.

The production of tyramine in the three lots of cheese ripened at three different temperatures is given in table 2 and the flavor development in table 3. The tyramine content of cheese made from pasteurized milk with commercial lactic starter increased only slightly with ripening up to one year (table 2). The cheese milk and starter were not contaminated with bacteria that produced tyramine as indicated by the low amount of this compound in the cured cheese. Consequently, at 50° F. the ripened cheese contained only a little more tyramine than cheese ripened at 40° F., and only slightly less tyramine than cheese ripened at 60° F. All of the cheese made with commercial starter only was mild in flavor after 4 months at 40, 50 and 60° F., but after 6 months' curing at 50 and 60° F. the cheese possessed a flavor of medium intensity, even though the tyramine content was low (table 3). The flavor was not typically good Cheddar, but was flat and the cheese at 60° F. was definitely overcured.

TABLE 2
Influence of temperature of ripening upon the production of tyramine in American Cheddar cheese made from pasteurized milk

Starter used in cheese	Days ripened										
	0	3	15	30	60	90	120	150	180	240	365
	Tyramine (γ/g)										
Ripened at 40° F.											
Lactic	5				7	8	7	6	3	6	3
<i>S. faecalis</i>	6				17	12	12	22	18	24	47
Lactic- <i>faecalis</i>	14				85	57	45	77	85	120	312
Ripened at 50° F.											
Lactic	5				14	12	12	12	12	10	17
<i>S. faecalis</i>	6				72	46	38	64	108	113	281
Lactic- <i>faecalis</i>	14				126	163	216	280	428	534	1,369
Ripened at 60° F.											
Lactic	5	13	14	12	19	14	16	14	17	28	37
<i>S. faecalis</i>	6	27	22	45	83	90	157	220	315	379	811
Lactic- <i>faecalis</i>	14	42	70	120	356	477	762	894	1,172	1,425	2,554

with a slightly burnt or caramelized taste. This interfered with judging the intensity of Cheddar flavor.

The cheese made with *S. faecalis* starter alone produced very little tyramine at 40° F. and the flavor remained mild for 6 months. When ripened at 50° F., the tyramine content of the cheese was 108 γ per g. and the flavor was medium+. The cheese ripened at 60° F. contained 315 γ per g. and the flavor was considered to be medium+ in intensity.

The cheese made with both lactic and *faecalis* starters contained 85 γ per g. after 6 months at 40° F. and the flavor was mild+. Ripened at 50° F. for 6 months, the cheese contained 428 γ per g. of tyramine and the flavor was medium+. The cheese ripened at 60° F. contained 1,172 γ per g. and was sharp – in flavor intensity. The flavor was typical Cheddar of excellent quality.

Too few temperatures of ripening were employed to permit any consideration

TABLE 3

Influence of temperature of ripening upon the development of flavor in American Cheddar cheese made from pasteurized milk

Starter used in cheese	Flavor score and intensity in cheese					
	Ripened 60 d.		Ripened 120 d.		Ripened 180 d.	
	Score	Intensity	Score	Intensity	Score	Intensity
Lactic	38.5	mild –	Ripened at 40° F.		39.0	mild
<i>S. faecalis</i>	38.2	mild –	38.5	mild –	39.0	mild
Lactic- <i>faecalis</i>	39.7	mild	39.2	mild	39.0	mild
			39.5	mild	40.0	mild +
Lactic	39.0	mild –	Ripened at 50° F.		39.0	medium –
<i>S. faecalis</i>	40.0	mild	39.0	mild +	39.7	medium +
Lactic- <i>faecalis</i>	40.5	mild +	39.0	medium	40.5	medium +
			40.0	medium +		
Lactic	39.5	mild +	Ripened at 60° F.		38.0 ^a	medium ^a
<i>S. faecalis</i>	40.0	medium	38.5	mild +	38.7	medium +
Lactic- <i>faecalis</i>	40.5	medium	39.2	medium +	39.5	sharp –
			40.5	sharp –		

^a Overcured with a slight burnt or caramelized flavor, so the intensity of Cheddar flavor was difficult to judge.

of trends in the increase of tyramine production with increased ripening temperatures. However, the tyramine content of the cheese showed an approximate direct logarithmic relation to the time of ripening in days (fig. 1). The average data (fig. 1) omit all data for cheese with lactic starter only, together with all cheese stored at 40° F., as the rate of increase of tyramine was too slow to show any trend.

The data on the volatile acidity, water-soluble protein, and pH are not presented as they duplicate some previously published data (2). There was very little increase in volatile acidity in 6 months at 40° F., and this slight increase was least for the *faecalis* cheese. After 6 months the volatile acidity of the cheese was 19 ml. 0.1 *N* acid per 100 g. at 40° F. and 42 ml. at 60° F. for lactic starter cheese, which compared with 18 and 41 ml. 0.1 *N* acid per 100 g. for

lactic-faecalis cheese. The water-soluble protein increased about three-fold in 6 months at 40° F. The effect of increased temperature of ripening on water-soluble nitrogen after 6 months showed the lactic starter cheese contained 4.4 per cent at 40° F. and 8.1 per cent at 60° F., and the *lactic-faecalis* cheese contained 4.3 and 8.4 per cent, respectively, of water-soluble protein.

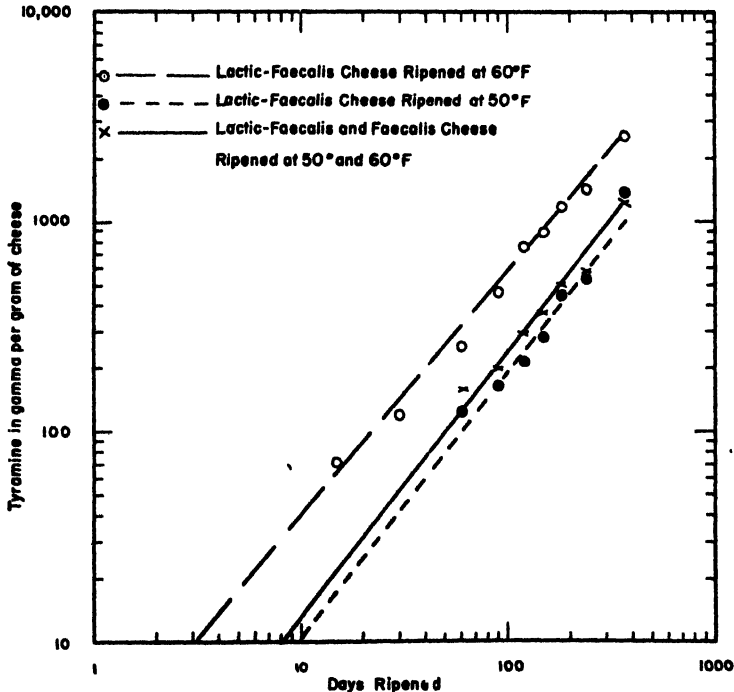


FIG. 1. The rate of tyramine production in cheese made with *S. faecalis* starter alone, and with commercial lactic and *faecalis* starters.

DISCUSSION

The amount of tyramine in the cured cheese made from pasteurized milk with commercial lactic starter alone was especially low as compared with the tyramine previously found in commercial Cheddar cheese (6). Undoubtedly, this pasteurized milk of high sanitary quality did not contain tyramine-producing bacteria or they may have been present in very small numbers. The cheese developed normal acidity, volatile fatty acids, and water-soluble protein during ripening, but the intensity of flavor remained mild and was not increased greatly by warmer ripening temperatures. This illustrates indirectly previous results (3) that increased tyramine and Cheddar flavor intensity are related. Limited flavor developed with ripening and with very little tyramine production, which shows that all flavor is not associated with tyramine, but most of the typical Cheddar flavor is related to the presence of tyramine.

S. faecalis alone in cheese increased tyramine production, but the effect was

very limited at 40° F. It should be observed that the temperatures of this refrigerator varied from 35 to 42° F. and was generally slightly below 40° F. *S. faecalis* grows poorly at this temperature. Ripening at 50 and 60° F. gave marked increases in tyramine and obvious increases in the intensity and quality of flavor. *S. faecalis* grows well at these warmer temperatures.

The use of regular lactic and *faecalis* starters in pasteurized milk markedly increased tyramine production in cheese at all temperatures of curing, including 40° F. The intensity of Cheddar flavor and the quality of the Cheddar flavor were best in the cheese made with both starters.

It should be pointed out that the percentage of starter added to the milk in these experiments was rather large, but similar results have been observed with variations from 0.1 to 2.0 per cent of the *faecalis* starter.

On several occasions it has been observed that Cheddar cheese made with *S. faecalis* starter not only developed flavor more rapidly but this flavor was maintained for longer periods of time than in cheese without this culture. This was observed in cheese cured at 60° F. The cheese made with lactic starter alone decreased progressively in score with increased age and the flavor intensity increased slightly. At 180 days this cheese was overcured and had an off-flavor. The cheese made with both lactic and *faecalis* starters was sharp—in intensity at 120 days, yet it was held for 180 days without developing an off-flavor. The best keeping quality was shown by the cheese made with both starters.

It is believed that 40° F. or below is too cold a temperature for ripening Cheddar cheese made from pasteurized milk of good quality with both starters to obtain maximum development of good Cheddar flavor in a reasonable period of time. An initial curing for 2 months at 60° F. or 4 months at 50° F. will develop good pronounced Cheddar flavor and then the cheese properly made may be stored a long time at 40° F. or below with assurance of excellent flavor.

In considering the data on flavor, it should be borne in mind that flavor intensity was rated over a 6-month period. Consequently, there is more chance for variation in judgement than if all samples were rated at one time.

This research emphasizes that the value of *S. faecalis* starter in ripening cheese will be greatest in milk of highest sanitary quality. Its value will be least in milk of low bacterial count but relatively high in normal infection with *S. faecalis* or other tyramine-producing bacteria, and in milk of lower sanitary quality which normally contains these bacteria in substantial numbers. When these bacteria were absent, force curing at a high temperature, such as 60° F., accelerate flavor development to some extent but not enough to obtain a full Cheddar flavor.

There is an indication that the rate of development of tyramine in the early days of ripening might be used as an index to predict whether cheese will cure slowly or rapidly. More data are necessary to establish this point.

CONCLUSIONS

The pasteurized milk used for making the experimental American Cheddar cheese was practically free from tyramine-producing bacteria, as indicated by

the tyramine content. After 6 months ripening at 40, 50 and 60° F., the tyramine content of cheese made with commercial lactic starter was only 3, 12 and 17 γ per g. The flavor was mild, medium – and medium in intensity and lacked good Cheddar characteristics.

When *S. faecalis* starter (special rapid acid-producing strain) was used alone in the pasteurized milk, the tyramine content of the cheese after 6 months ripening at 40, 50 and 60° F. was 18, 108 and 315 γ per g. The flavor intensities were mild, medium – and medium +, respectively.

The use of both commercial lactic and *S. faecalis* starters in the pasteurized milk produced the greatest development of tyramine and good, typical Cheddar flavor. After 6 months' ripening at 40, 50 and 60° F. the tyramine content was 85, 428 and 1172 γ per g. and the flavor intensity was mild +, medium + and sharp –. The sharp – intensity of flavor was obtained in 4 months.

Cheddar cheese containing *S. faecalis* developed more tyramine and Cheddar flavor as the ripening temperatures were increased. It maintained its good flavor for a longer period of time without becoming overcured at warm temperatures than did cheese made only with ordinary lactic starter. The increase in the tyramine content of cheese showed an approximately direct logarithmic relation to the days ripened.

REFERENCES

- (1) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Flavor, Volatile Acidity, and Soluble Protein of Cheddar and Other Cheese. *J. Dairy Sci.*, 30: 165–174. 1947.
- (2) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Development of Flavor in American Cheddar Cheese Made from Pasteurized Milk with *Streptococcus faecalis* Starter. *J. Dairy Sci.*, 31: 275–284. 1948.
- (3) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Relationship of the Amount of Tyramine and the Numbers of *Streptococcus faecalis* to the Intensity of Flavor in American Cheddar Cheese. *J. Dairy Sci.*, 31: 305–314. 1948.
- (4) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Rapid Direct-distillation Method for Determining the Volatile Fatty Acids of Cheese. *J. Dairy Sci.*, 29: 861–871. 1946.
- (5) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Growth and Survival of *Streptococcus faecalis* in Pasteurized Milk American Cheddar Cheese. *J. Dairy Sci.*, 31: 285–292. 1948.
- (6) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Tyramine Content of Cheese. *J. Dairy Sci.*, 31: 293–303. 1948.
- (7) WILSON, H. L. Making American Cheddar Cheese of Uniformly Good Quality. U. S. Dept. of Agr., B. D. I. M. 947. Nov., 1942.

EFFECT OF THYROXINE ON FERTILITY OF BOVINE SEMEN¹

A. B. SCHULTZE AND H. P. DAVIS

Department of Dairy Husbandry, University of Nebraska, Lincoln

The addition of proper amounts of D,L-thyroxine to bovine semen appears to exert an influence on sperm metabolism (1, 2). It is logical to suppose that a change in metabolism influences semen fertility. Therefore this study was undertaken to determine whether bovine semen fertility is influenced by the addition of thyroxine. Since the over-all fertility of bovine semen obtained under artificial breeding conditions depends both on the initial fertility and the maintenance of a high level of fertility during storage, the data were studied to determine whether thyroxine influences the initial fertility, the maintenance of fertility or both of these characteristics.

METHODS

D,L-thyroxine was added to egg yolk-phosphate-buffered bovine semen in a concentration of 10 γ per 100 ml. diluted semen. A stock solution of the thyroxine was prepared by weighing 10 mg. of the material and dissolving it slowly and completely in distilled water by the addition of 0.1 N NaOH drop by drop. When dissolved the solution was made up to 100 ml. volume. The thyroxine solution added to the diluted semen was prepared from this stock solution by adding phosphate buffer in measured volumes so that the buffered thyroxine solution contained 5 γ thyroxine per ml. All thyroxine solutions were stored at 40° F. Portions of this latter buffered solution were added to the egg yolk-phosphate-buffered semen so that the final concentration of thyroxine was 10 γ in 100 ml. diluted semen.

Semen samples were selected at random from 18 bulls in the University stud and treated with thyroxine. Other semen samples from the same bulls during the same periods of time served as controls. Non-return rates obtained from the use of such a series of semen samples vary from that obtained with another series even where semen are from the same bulls during the same season. The variation among several such series involving semen sample numbers and service numbers approximating those of the thyroxine-treated semen in our experiments appeared not to exceed 2 percentage units in non-return rate. To compare the results of treatment on the same semen sample, an experiment was carried out in which one portion of a semen sample was treated with thyroxine and sent to one group of associations for use; the other portion, untreated, was sent to a second group of associations. The two association groups had similar non-return rates when both used untreated semen from the same bulls.

Breeding results are based on 5 months' non-return rates of first and second services in routine artificial breeding.

Received for publication November 13, 1948.

¹ Published with the approval of the Director as Paper No. 462, Journal Series, Nebraska Agricultural Experiment Station.

RESULTS

The experiment to determine the effect of thyroxine on fertility of bovine semen was carried out over a 2-year period. In the first part of the experiment a portion of randomly selected semen samples was treated with thyroxine and sent to group 1 associations for use. The other portion of these semen samples was left untreated and sent to group 2 associations. More than 50 individual semen samples were used in this part of the experiment. The results showed that the non-return rate for the treated semen in group 1 (422 services) was 59.1 per cent and that for the untreated portion of the samples in group 2 associations (456 services) was 54.4 per cent, a difference of 4.7 percentage units. However, when the semen sent to both groups 1 and 2 was all untreated and from the same bulls, the non-return rate for the group 1 associations was 1.2

TABLE 1
Non-return rate with treated and control semen with age

	Days storage								All days combined	
	1		2		3		4			
	No. services	5-mo. non-returns	No. services	5-mo. non-returns	No. services	5-mo. non-returns	No. services	5-mo. non-returns	No. services	5-mo. non-returns
	(%)		(%)		(%)		(%)		(%)	
Semen treated with thyroxine	384	65.8	821	66.1	496	62.5	166	60.2	1887	64.6
Control semen	1303	63.1	2470	59.5	1489	56.0	667	52.1	5929	58.5
Increase over control	2.7		6.6		6.5		8.1		6.1	

percentage units lower than for the group 2 associations. Therefore, there appeared to be a net increase of 5.9 percentage units in non-return rate resulting from the presence of thyroxine in the semen.

The experiment was extended by sending semen samples treated with thyroxine to all associations. The non-return rate obtained with treated semen was compared with that obtained with control semen from the same bulls during the months in which semen was treated. The samples used as controls were from those used before or after the treated samples. The non-return rate from 92 treated semen samples with 1,867 services involved was 64.6 per cent. The non-return rate from untreated control semen samples was 58.5 per cent. Thus the addition of thyroxine appeared to increase the non-return rate by 6.1 percentage units.

A comparison of the non-return rates from treated and control semen with days of storage is shown in table 1. It is apparent that fertility was maintained

TABLE 2

Comparison of non-return rate of thyroxine-treated and control semen by individual bulls

Semen samples	Bull	Treated		Control		Difference
		Services	5-mo. non-returns	Services	5-mo. non-returns	
	(No.)	(No.)	(%)	(No.)	(%)	(Percentage Units)
1	5	66	56.1	136	52.2	+ 3.9
2	2	36	75.0	112	52.7	+ 22.3
3	6	95	47.3	307	50.2	- 2.9
4	6	91	62.6	228	57.5	+ 5.1
5	1	20	85.0	198	60.1	+ 24.9
6	9	201	62.7	347	60.8	+ 1.9
7	7	235	65.1	463	59.8	+ 5.3
8	4	128	66.4	179	58.1	+ 8.3
9	5	97	63.9	231	54.1	+ 9.8
10	6	180	65.6	254	60.4	+ 5.2
11	1	22	63.6	193	59.5	+ 4.1
12	7	80	68.7	200	56.5	+ 12.2
13	13	185	66.5	391	60.1	+ 6.4
14	6	76	68.4	268	64.9	+ 3.5
15	1	35	62.8	193	67.4	- 4.6
16	2	40	75.0	89	68.5	+ 6.5
All	81	1587	64.5	3789	58.8	+ 5.7

* Bulls having less than 20 treated services omitted from table.

at a higher level throughout the 4 days of use. The difference during the first day of use is less than with increased storage, indicating a delayed thyroxine effect.

The data were studied by grouping semen samples by individual bulls. The comparison is shown in table 2. Semen samples from bulls used in less than 20 services have been omitted from the tabulation. In evaluating the average

TABLE 3

Comparison of non-return rate with thyroxine-treated and control semen by individual breeding associations. (Only services with same storage time for control and treated semen considered.)

Association	Treated		Untreated		Difference
	No. services	5-mo. non-returns	No. services	5-mo. non-returns	
		(%)		(%)	(Percentage Units)
A	320	60.3	308	55.8	+ 4.5
B	256	65.6	264	61.7	+ 3.9
C	86	69.8	114	56.9	+ 12.9
D	186	39.0	132	35.6	+ 3.4
E	98	73.5	99	61.5	+ 12.0
F	122	62.3	146	55.8	+ 6.5
G	107	60.7	95	53.6	+ 7.1
H	74	82.4	79	69.6	+ 12.8
I	115	73.0	121	63.6	+ 9.4
J	249	63.9	250	55.8	+ 8.1
All associations	1563	63.4	1608	56.7	+ 6.7

non-return rate for all bulls, the non-return rate percentage of each bull was weighted in proportion to the number of treated services so that the percentage influence of each bull's services on the total results would be the same for both treated and untreated semen. On this basis of comparison, thyroxine increased the conception rate 5.6 percentage units. The semen from two bulls showed a decrease in fertility due to treatment; however, in one of these bulls only one semen sample represented the treated services.

In a comparison of treated and control semen by individual associations, the controls were recorded only for those days on which the treated semen was used, to eliminate insofar as possible the effect of semen storage on the comparison. Results are shown in table 3. The non-return rate in all associations was increased by treatment and the difference for all associations was found to be 6.7 percentage units. This percentage unit increase is somewhat higher than that found with other bases of comparison; however, it is not significantly different.

To test whether the difference in non-return rate due to treatment was statistically significant, the results were analyzed on an individual semen sample basis for both treated and control samples. Semen samples with less than ten services were eliminated. The non-return rate percentages were converted to angles. The difference between treated and control samples was found to be significant at less than the 5 per cent level. When the variance between individual semen samples was eliminated, significance was at less than the 1 per cent level.

DISCUSSION

It appears from the data at hand that 10 γ per cent D,L-thyroxine when added to diluted bovine semen increases the fertility. This is apparent with semen from bulls of average or above average fertility. The difference in fertility is small for the first day of storage but increases to a maximum the second day of storage. This increase in fertility apparently is maintained for a 4-day storage period. The non-return rate for treated semen was lower than that for control semen with the semen of one bull. This bull's average fertility was relatively low and it is possible that thyroxine in the amount used had a detrimental effect on this poor semen. A limited number of thyroxine-treated semen samples from bulls with less than 50 per cent non-return rate was available for study. It seems from these limited data that semen low in fertility is increased in non-return rate above that of control semen of low fertility during at least 2 days of storage, but declines below that of the control level by the 3rd or 4th day of storage. Semen lower in fertility than that represented by the data at hand may be more unfavorably affected by storage.

The selection of semen samples used for controls during the period of the experiment was varied so that accidental selection of better than average or poorer than average semen from the bulls on experiment would be evident if this were affecting the results. The selection of different control semen samples resulted in a difference of 2 percentage units in the control values. This is a non-significant difference and can probably be expected by random selection of

semen from the same bulls during the same periods of the year with the number of services and semen samples involved.

SUMMARY

Ten micrograms per cent of D,L-thyroxine was added to diluted bovine semen. This semen was used in artificial breeding.

All treated services (2,289) averaged 5.7 to 6.7 percentage units higher in percentage of non-returns to service after 5 months than untreated semen from the same bulls collected during the same months that the treated semen was used. In a comparison of either individual bulls or individual breeding associations, an increase in non-return rate is also apparent with thyroxine-treated semen.

The increase in fertility with the addition of thyroxine appears to be maintained for a 4-day storage period with semen of average or above average fertility.

REFERENCES

- (1) SCHULTZ, A. B., AND DAVIS, H. P. Some Effects of Adding Thyroxine to Bovine Semen. *J. Dairy Sci.*, 30: 543-544. 1947.
- (2) SCHULTZ, A. B., AND DAVIS, H. P. Effect of Thyroxine on Oxygen Consumption of Bovine Spermatozoa and Semen. *J. Dairy Sci.*, 31: 946-950. 1948.

THE EFFECT OF TESTIS BIOPSY ON SEMEN CHARACTERISTICS OF BULLS¹

J. F. SYKES, T. R. WRENN, L. A. MOORE, P. C. UNDERWOOD, AND
W. J. SWEETMAN

*Bureau of Dairy Industry, Agricultural Research Administration,
United States Department of Agriculture*

Biopsy technics have been used by many investigators to study histological changes in various living organs. Erb *et al.* (2) in a preliminary report have described a biopsy technic by means of which they correlated semen quality with testis morphology in dairy bulls during vitamin A studies. We have used this same technic in experiments on the effects of energy restriction on semen characteristics of bulls, and during the course of these experiments evidence was obtained that this biopsy technic produced changes in semen characteristics and in testis morphology which were unrelated to dietary conditions.

MATERIALS AND METHODS

Studies were made on three mature bulls. When the first biopsy specimen was taken, all were just past 6 years of age. Two of the bulls (no. 1 and 2) had been on a ration which restricted energy intake to 65 per cent of requirement, up to the time the first specimen (from the left testis only) was taken. Feed intake then was raised to 100 per cent of requirement and maintained at that level until the bulls were disposed of. A specimen was obtained (from the right testis) on one of these bulls (no. 2) 6.5 months later and on the other (no. 1) 10.5 months later. Both bulls were castrated 3 months after the second biopsy and representative sections of testis tissue were obtained.

The first specimen (from the left testis) was obtained from the third bull (no. 3) immediately before the ration was reduced to the 65 per cent energy-intake level. He remained on this level of energy-intake for 15 months, and a second specimen (from the right testis) then was obtained. Feed intake then was increased to 100 per cent of requirements, and the bull was castrated 3 months later.

The biopsy technic performed under local anesthesia was essentially that described by Erb *et al.* (2), with the exception that hemorrhage always was controlled by applying manual pressure to the spermatic artery until a solid clot had formed. The tunica albuginea and skin incision always were sutured. Recovery was rapid and uneventful in every instance.

Semen was collected from the bulls by means of an artificial vagina. With only occasional omissions, four ejaculates were collected weekly from each bull during the period of observation, two ejaculates being obtained in succession at each of two services. Motility determinations were made on undiluted semen

Received for publication November 29, 1948.

¹ This work was supported in part by an appropriation from Bankhead-Jones special research funds.

in a stage warming apparatus. Sperm counts and the determination of abnormal spermatozoa were made with a hemocytometer on fresh semen diluted with a 0.9 per cent saline solution. The data presented here all are based on the second ejaculate.

Tissue sections were stained with hematoxylin and eosin for general morphological examination and with Mallory's aniline blue connective tissue stain to detect the relative amounts of connective tissue in the various specimens.

RESULTS

The data obtained on semen characteristics are shown for each of the three bulls in table 1. It will be noted that each biopsy produced a marked decrease

TABLE 1
Changes in sperm characteristics as the result of biopsy

Period of observation	Sperm count	Initial motility	Storage motility ¹	Percentage of abnormal sperm cells	Volume	Number of observations
	(millions/ml.)	(%)	(%)		(ml.)	
<i>Bull no. 1</i>						
Control (60 days)	1360	54.3 ²	9.9 ^a	63.9 ^a	3.4	
Biopsy specimen obtained—returned to adequate feed intake						
(60 days)	700	85.0	17.5	33.5	3.8	4
(274 days)	639	67.9	27.6	34.0	5.0	27
Second biopsy (no change in feed)						
(30 days)	653	30.3	7.0	37.0	4.4	3
(60 days)	388	44.8	8.8	28.2	5.8	8
<i>Bull no. 2</i>						
Control (60 days)	1012	70.0	40.0	27.7	2.8	9
Biopsy specimen obtained—returned to adequate feed intake						
(60 days)	862	72.5	25.5	24.6	4.2	5
(142 days)	556	73.0	30.8	20.7	4.4	13
Second biopsy (no change in feed)						
(30 days)	723	38.3	20.0	45.0	4.6	3
(60 days)	284	46.3	11.9	32.6	4.2	9
<i>Bull no. 3</i>						
Control (60 days)	841	86.2	48.1	18.9	4.7	8
Biopsy specimen obtained—feed intake reduced						
(50 days)	458	74.5	26.5	41.0	5.5	5
(376 days)	465	76.0	31.7	38.8	4.3	41
Second Biopsy—returned to adequate feed intake						
(30 days)	125	5.0	2.0	75.2	2.7	4
(60 days)	0.13	0	0	82.0	3.7	7

¹ Storage motility = motility after 48 hrs. storage at 4–6° C.

² These low motility values and the high percentage of abnormal sperm cells resulted from restriction of feed intake.

in sperm concentration in all three bulls, which roughly amounted to 50 per cent except in the case of the second biopsy on bull no. 3. Sperm production practically ceased following the second biopsy in the case of bull no. 3. The

full effect of a biopsy was not always immediately evident, and for this reason the period of observation following each biopsy has been divided into a short and a longer interval. Usually the first two or three ejaculates obtained contained numbers of sperm characteristic of the pre-biopsy period. This was at least partly due to the fact that a 2-week period of sexual rest was usually allowed after each biopsy. The decrease in sperm concentration which followed this short interval persisted throughout the remainder of the observation period in all cases, and there has been no indication that improvement in spermatogenesis occurred even though the periods of observation after the first biopsy was performed ranged from 202 to 426 days.

It also will be noted that changes were made in the ration of these animals in every instance at the time the first biopsy specimen was obtained. In the case of bull no. 3, feed intake was reduced at this time. During the following 14-month period, his weight loss amounted to 293 lb. The change in sperm concentration possibly could be accounted for by the poor nutritional condition of this bull. However, a much greater decrease in sperm concentration occurred in the semen of this bull when the second biopsy was performed, even though feed intake was increased to normal at this time. Moreover, this change was noted in the semen of bulls no. 1 and no. 2 after both biopsies. The first biopsy was performed on these bulls at a time when feed intake was increased and the bulls thereafter were improving in condition. The second biopsy was performed on these bulls while they were on normal and constant feed intakes. The change in sperm count was just as great as under the previous regime. It seems evident, therefore, that the change in sperm concentration was related to the biopsies rather than to changes in the ration which coincided with the biopsies in most instances.

It also is evident that a serious misinterpretation of experimental results might have occurred if all three of these bulls had been biopsied just previous to the time that the restriction in energy intake was imposed. It would not have been illogical to ascribe the decrease in sperm production to the reduced energy intake rather than to the effects of biopsy. The data presented by Erb *et al.* (2), in the paper in which they described the biopsy technic, show that a marked drop in concentration of spermatozoa occurred after each of the biopsies that were taken from the bull used in their work. The decrease in concentration of spermatozoa after the first biopsy was ascribed to a gradual depletion of stored sperm. No comment was made on the decrease after the second biopsy.

In view of our results, it would appear that the semen change reported by Erb *et al.* (2) was related more to the biopsies than to the vitamin A status of the animal. It also will be noted that improvement in semen occurred following the initial drop in sperm concentration after each biopsy on the bull described by Erb *et al.* (2). This effect has not been seen in the present work and this difference in results between the two studies may be due to the differences in age of the bulls used.

The effect of the biopsies on motility and on the percentage of abnormal cells was somewhat variable. The first biopsy on bulls no. 1 and 2 did not decrease motility or increase the number of abnormal spermatozoa. The increase in motility and the decrease in abnormal cells which occurred after the first biopsy, in the case of bull no. 1, is believed to be due to the improved nutritional status of the bull during this interval. Attention is drawn to the fact that the control values for this bull (see table 1) were abnormal and a result of the previous feed restriction. In the case of bull no. 3, however, the first biopsy did decrease motility and increase the percentage of abnormal cells. The fact that no pro-

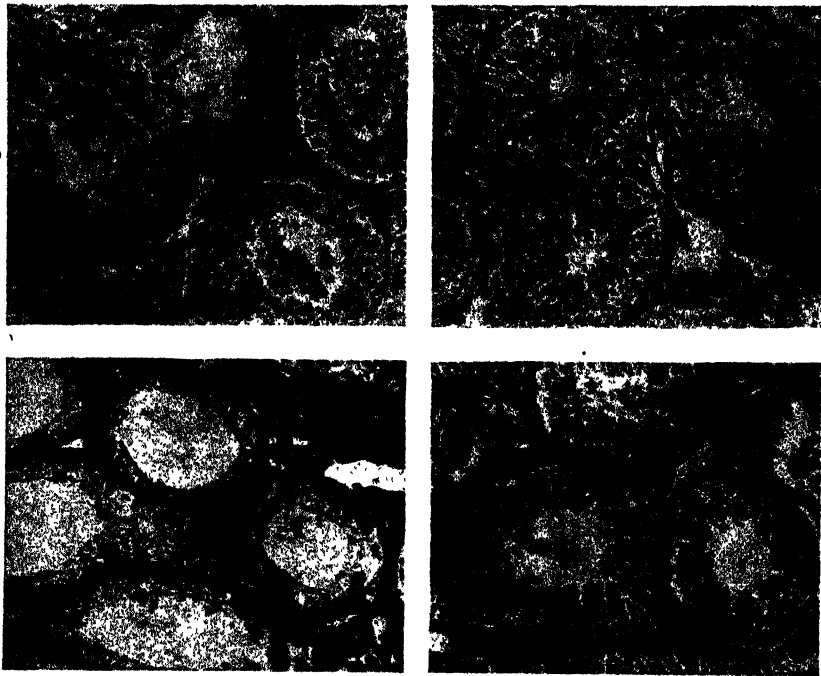


FIG. 1. Comparison of biopsy specimens of testis and specimens obtained on the same testis after castration. X200.

- 1A—Right testis biopsy specimen.
- 1B—Right testis at time of castration.
- 1C—Left testis biopsy specimen.
- 1D—Left testis at time of castration.

gressive change in these characteristics occurred in the succeeding period of feed restriction would indicate this was an effect of the biopsy and not a result of feed restriction. In all cases, however, the succeeding biopsy on the opposite testis markedly reduced motility and increased the percentage of abnormal cells. The data therefore would indicate that a single biopsy on one testis may or may not affect the motility and normality of the sperm cells but that a second biopsy, even when obtained from the opposite testis, has a definite and adverse effect on the motility and normality of sperm cells. Bogart and Mayer (1) noted that

similar changes in semen characteristics occurred in sheep after removal of one testis. Moore (3) also has noted that adhesions occur around the testis of bulls following inflammation due to infection or trauma of the testis and are accompanied by a progressive deterioration of semen quality. In the three bulls in the present study, marked adhesions between the tunica vaginalis and tunica albuginea were noted when the bulls were castrated.

The photomicrographs shown in figure 1 are representative of the morphological changes noted in the testis tissue as determined at castration. A comparison of figure 1A and 1B illustrates the marked change which occurred in the right testis following biopsy. Figure 1A, from tissue taken at biopsy, shows there was some disorganization and desquamation of tissue and that sperm cells were not numerous in the tubules. Figure 1B shows the condition of the same testis 3 months later. Sperm cells were practically nonexistent and spermatogenic tissue was reduced markedly. This tissue, which was obtained at castration, was representative of the general condition throughout the testis except in the case of bull no. 3. In this bull, while spermatogenesis was reduced, less than 25 per cent of the tubules showed this marked degeneration, although most tubules showed degenerative changes when compared to the biopsy specimens obtained previously.

Figure 1C represents the biopsy specimen and figure 1D the tissue obtained at castration from the left testis. It will be noted that there is little or no difference in the appearance of the tissues. Two of the bulls (no. 1 and 2) reacted in this manner. The left testis of the third bull, however, showed very marked degeneration at the time of castration. The testis was about $\frac{1}{3}$ normal size and the upper third was distinctly calcified. Histologically, degeneration as marked as that shown in figure 1B was present throughout the testis and the tubules in the calcified area were filled with mineral deposit.

It is difficult to account for the variability in reaction to biopsy between the right and left testis of these animals. In all cases the interval of time between biopsy of the right testis and castration was 3 months. The intervals between biopsy of the left testis and castration were 14, 10 and 17 months for bulls no. 1, 2 and 3, respectively. In the case of bulls no. 1 and 2, the presence of marked degeneration in the right testis 3 months after biopsy and little or no change in the left testis 10 or 14 months after biopsy might indicate that repair had occurred in the left testis during the longer time interval. If repair did occur in the testis of these two bulls, it was unaccompanied by any improvement in spermatogenesis as previously noted. The marked degeneration noted in the left testis of bull no. 3 at castration, even though an interval of 17 months elapsed between biopsy and castration, is in direct contrast to the other two bulls. Bull no. 3 lost considerable weight (293 lb.) during 14 of the 17 months of this interval of time due to restriction of feed intake, whereas bulls no. 1 and 2 had adequate feed and were gaining in weight throughout most of this period. It therefore is possible that the reduced feed intake of bull no. 3 intensified the biopsy effect and was responsible for the difference in effects on the left testis observed between this bull and bulls no. 1 and 2.

As mentioned previously, rather extensive adhesions between the tunica vaginalis and tunica albuginea were found at castration. Marked connective tissue proliferation, of course, was present at the site of biopsy in every instance. In addition to these changes, an increase in the amount of connective tissue throughout the intertubular spaces of both testes was noted in the tissues obtained when the bulls were castrated. This reaction was not as intense as that observed at the biopsy site, but in every instance a distinct increase was noted in the amount of intertubular connective tissue as compared with the amount in the original biopsy specimens. This increase in connective tissue was roughly

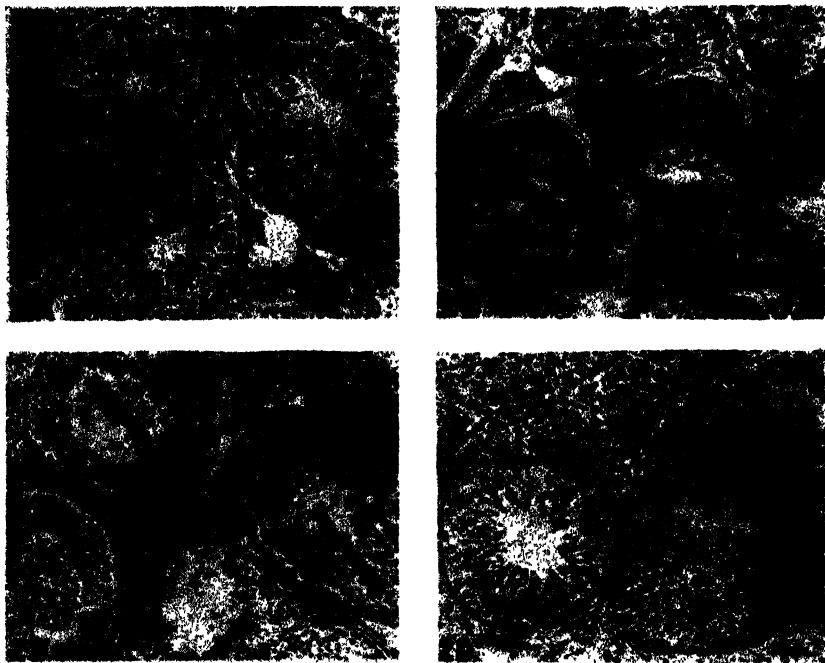


FIG. 2. Comparison of right and left testis biopsy specimens from bulls no. 2 and no. 3. X200.

- 2A—Left testis specimen (bull no. 2).
- 2B—Right testis specimen (bull no. 2).
- 2C—Left testis specimen (bull no. 3).
- 2D—Right testis specimen (bull no. 3).

proportional to the degree of tubular degeneration observed. It was greatest in the left testis of bull no. 3 and in the right testis of bulls no. 1 and 2 in which the greatest tubular change was observed and, while definitely increased, was less evident in the right testis of bull no. 3 and the left testis of bulls no. 1 and 2. It therefore would appear that the biopsies produced a marked proliferation of connective tissue throughout the testis and that this change was related to the morphological and functional changes which were observed in the tubules.

If the increased collagen noted in these sections was a result of a fibroblastic

reaction, this reaction had subsided by the time sections were obtained. However, there was some evidence that the numbers of interstitial cells were increased in those specimens which showed increases in connective tissue.

The evidence at our disposal did not suggest that a biopsy on one testis had any marked effect on the opposite testis. A comparison of tissues obtained at successive biopsies of the left and right testis is shown in figure 2 to illustrate this point. Tissue from the left testis biopsy (2A) and tissue from the right testis (2B) obtained by biopsy 6 months later from bull no. 2 is shown. Comparison of these tissues indicates that possibly some change occurred in the right testis as a result of biopsy on the left testis. There also was some slight indication of an increase in connective tissue in the right testis. A comparison of figures 2C and 2D, which are similar tissues from bull no. 3, shows no difference. A like comparison between the biopsy specimens from the left and right testis from no. 1 indicated no effect other than some increase in connective tissue in the right testis at biopsy. The intervals between biopsies in the case of bulls no. 1 and 3 were 11 and 14 months, respectively, whereas it was only 6 months in the case of bull no. 2. If the difference noted between figures 2A and 2B really indicates that the biopsy of the left testis affected the opposite testis of bull no. 2, and that similar changes were produced in the right testis of bull no. 1 and bull no. 3, it might be assumed that the longer interval between biopsies in the case of the latter two bulls gave sufficient time for repair to take place so that no evidence of damage was present at the succeeding biopsy. In general, however, the direct evidence available would not indicate that biopsy on one testis produced morphological changes in the other testis.

SUMMARY AND CONCLUSIONS

1. Biopsy of the testis of mature bulls resulted in a marked decrease in sperm concentration in the semen.

2. Biopsy of a single testis had variable effects on motility or percentage of abnormal sperm, but a succeeding biopsy on the second testis decreased motility and increased the percentage of abnormal sperm, and in one bull resulted in almost complete absence of spermatogenesis.

3. Marked morphological changes were observed in the tubules of the testis and in the amount of intertubular connective tissue. In one instance, marked atrophy of the testis occurred as a result of biopsy.

4. These data would indicate that considerable care must be used in interpreting data in experimental studies on semen production and semen characteristics where biopsies are performed in conjunction with other nutritional or physiological technics.

REFERENCES

- (1) BOGART, RALPH, AND MAYER, DENNIS T. Environmental Temperature and Thyroid Gland Involvement in Lowered Fertility of Rams. Mo. Agr. Expt. Sta. Research Bull. 402. 1946.
- (2) EBB, R. E., ANDREWS, F. N., BULLARD, J. F., AND HILTON, J. H. A Technique for the Simultaneous Measurement of Semen Quality and Testis Histology in Vitamin A Studies of the Dairy Bull. J. Dairy Sci., 27: 769. 1944.
- (3) MOORE, G. B. Infertility in Bulls. J. Am. Vet. Med. Assoc., 112: 25. 1948.

SOME FACTORS INFLUENCING THE REDUCING SYSTEMS IN DRY WHOLE MILK¹

H. A. HARLAND, S. T. COULTER, AND ROBERT JENNESS

Divisions of Dairy Husbandry and Agricultural Biochemistry, University of Minnesota, St. Paul

The first extensive data relating the heat-labile sulfur of milk to cooked flavor and antioxygenic activity were obtained by Gould and Sommer (7) and Josephson and Doan (13). The former collected the heat-labile sulfur with lead acetate paper and found that heat treatments sufficient to cause cooked flavors and a lowering of the oxidation-reduction potential also produced volatile sulfur. Very similar results were obtained by Josephson and Doan (13) using the nitroprusside test for heat-labile sulfur.

The antioxygenic properties of dry whole milk manufactured from milk preheated to a high temperature have been ascribed to the sulfur-containing reducing groups (10, 11). Mattick *et al.* (15) were the first to show that the presence of nitroprusside-reducing substances resulting from the preheating treatment of the milk is associated with increased resistance to oxidation of the dry milk during storage.

The effect of several preheating temperatures on the keeping quality of the dry whole milk was studied by Findlay *et al.* (6). Preheating temperatures producing antioxygenic effects in the powder were those yielding positive nitroprusside and volatile sulfur tests. No data were reported concerning the effect of subsequent oxidation of the dry whole milk during storage on the sulphydryl groups initially present.

A method for the estimation of sulphydryl groups in blood based on reduction of thiamin disulfide has been adapted to milk by Harland and Ashworth (9). The experiments described herein were undertaken to test the thiamin disulfide reagent for the estimation of the antioxygenic qualities of dry whole milk, prepared from milk preheated to high temperatures, to determine the source of the groups that reduce this reagent and to compare the method with other means of measurement of reducing substances in milk.

METHODS

Thiamin disulfide reducing substances (TDRS) were determined essentially according to the method of Harland and Ashworth (9). Acid ferricyanide reducing substances (AFRS) were estimated by the Chapman and McFarlane procedure (2) as modified by Crowe *et al.* (4). Ascorbic acid (apparent) was estimated by the method of Doan and Josephson (5). Solubility indices of the dry whole milk were performed according to the method of the American Dry Milk Institute (1).

Received for publication November 29, 1948.

¹ Paper no. 2439, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

Drying of the skim milk and the whole milk used in this work was accomplished in an experimental spray dryer of the University of Minnesota. Milk flow was regulated to secure an exit air temperature of 85° C. for what was considered to be a normal drying temperature and 104° C. for a high drying temperature.

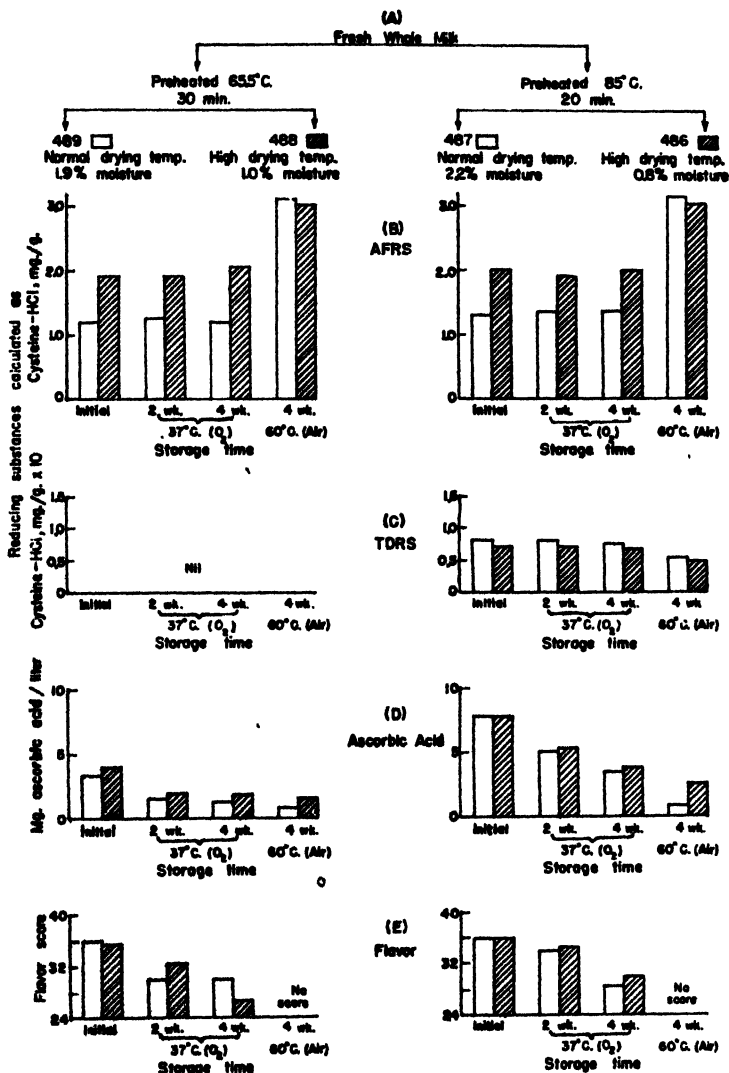


FIG. 1. The effects of preheating and drying temperatures on the acid ferricyanide (AFRS) and thiamin disulfide (TDRS) reducing substances, ascorbic acid, and keeping quality of dry whole milk. Similar results were obtained from a duplicate experiment.

Heat treatments of the liquid systems were done in thin-walled, 8-inch stainless steel test tubes of 20 ml. capacity immersed in a rapidly-circulating water

bath maintained at constant temperature. Deaeration of the liquid systems was accomplished by a brief period of boiling, under a pressure of approximately 25 mm. of mercury, and flooding the system with commercial nitrogen. This process was repeated three times before heating in an atmosphere of nitrogen.

Scoring of the dry whole milk was done according to the student score card.

Nonfat dry milk solids were prepared from freshly separated milk of good quality preheated at 63° C. for 30 minutes. Dry whole milk was prepared from good quality fresh milk and had solubility indices not exceeding 0.1. Heat treatments were as indicated in the data. Casein was prepared as previously reported (12) and dispersed in a minimum quantity of 0.1 *N* NaOH to pH 6.6 to obtain the sodium caseinate. Serum proteins were prepared by removal of the casein from reconstituted nonfat dry milk solids with acetate buffer at pH 4.5, concentration of the whey by freezing, exhaustive dialysis in Visking sausage casings and further concentration by pervaporation² from the casing. This preparation was stored under toluene at approximately 4° C. until used. Lactose was of U.S.P. quality.

RESULTS

Relation of reducing substances to retention of ascorbic acid and flavor during storage. A preliminary experiment was designed to indicate some of the re-

TABLE 1
Composition of the simplified systems used for heat treatment

System	Casein (as Na Caseinate)	Milk serum protein	Lactose	Phosphate buffer pH 6.6
	(g./100 ml.)	(g./100 ml.)	(g./100 ml.)	(μ)
1	2.50			0.10
2	2.50		4.75	0.10
3		0.70		0.10
4		0.70	4.75	0.10
5	Reconstituted nonfat dry milk solids 10 g./100 ml.			

lationships of reducing capacity to the conditions of manufacture and storage of dry whole milk and to determine whether retention of ascorbic acid and flavor during storage is related to reducing capacity. The experimental plan as well as the data are presented in figure 1.

The acid ferricyanide reducing substances (AFRS) (fig. 1B) were not influenced significantly by the preheating temperatures used, but were increased by the higher drying temperature. This is in agreement with results reported in a recent paper (4). Although storage of the powder at 37° C. for periods up to 4 weeks had little effect on the AFRS, storage at 60° C. caused a large increase in these substances.

The preheating temperature was the predominant factor influencing the thiamin disulfide reducing substances (TDRS) (fig. 1C). The apparent de-

² Evaporation of water from the sol through the membrane. (Kober, P. A., Pervaporation, Perstillation, and Percrystallation. J. Am. Chem. Soc., 39: 944-48. 1917.)

crease in these substances during storage of the dry whole milk at 60° C. for 4 weeks could not be substantiated when this experiment was repeated. High drying temperatures appear to depress slightly the TDRS produced in the milk by heat treatment.

The protective action of the higher preheating temperature on the ascorbic acid content of the dry whole milk both during drying and in subsequent storage is apparent in figure 1D. The high drying temperature also seems to afford some degree of protection for this easily oxidized substance.

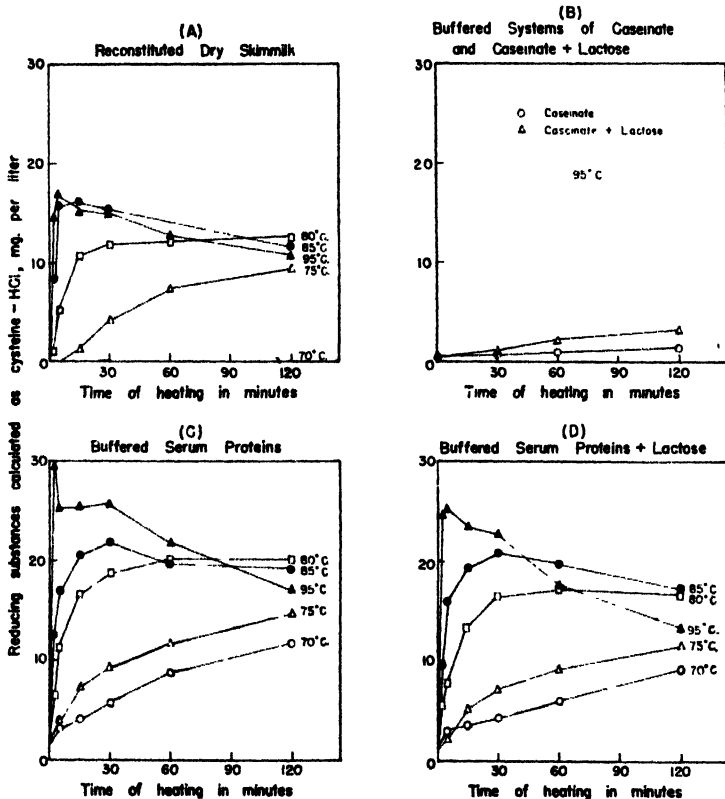


FIG. 2. The effect of heat treatment of skim milk and simplified systems on production of thiamin disulfide reducing substances. The points shown in these graphs are the average of at least two values.

The flavor scores (fig. 1E), although typically rather erratic, show some increased retention of quality during storage as the result of the higher preheating temperature.

Source of TDRS and AFRS produced during heat treatment. It is believed generally that the sulfhydryl groups liberated in milk during heat treatment originate from the proteins and, more specifically, the serum proteins. To obtain information on this point skim milk (actually reconstituted nonfat dry milk

solids) and various simplified systems, the composition of which is tabulated in table 1, were heat-treated at various temperatures for periods up to 120 minutes. Reducing power was determined in these systems by both the thiamin disulfide and acid ferricyanide methods. Since during preliminary trials the oxygen tension of milk had been found to affect the production of TDRS, the systems studied in this experiment were deaerated before beginning the heat treatments. The data for TDRS and AFRS are shown in figures 2 and 3, respectively.

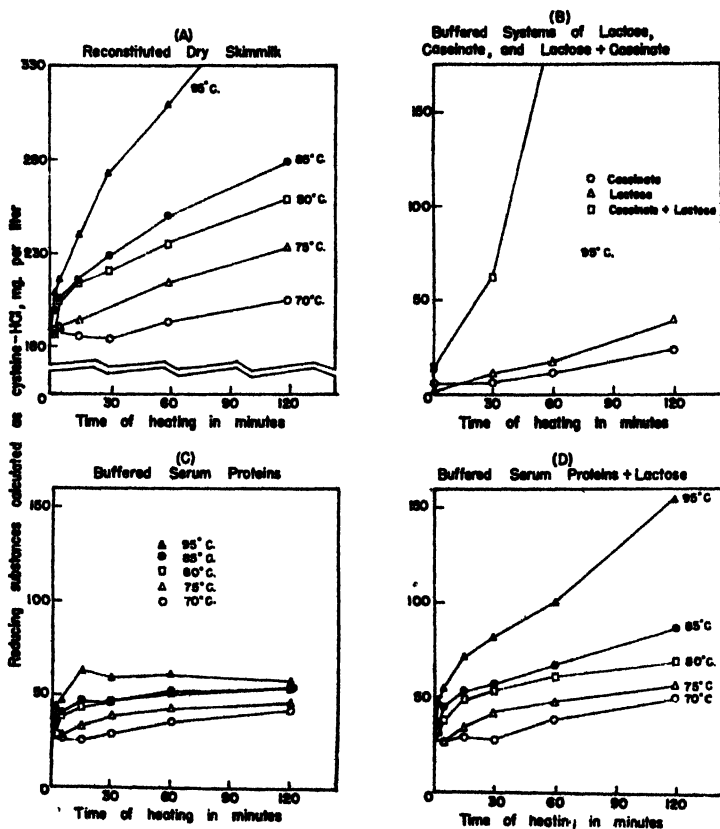


FIG. 3. The effect of heat treatment of skim milk and simplified systems on production of acid ferricyanide reducing substances. The points shown in these graphs are the average of at least two values.

For comparative purposes, skim milk was the first system studied (fig. 2A). It may be noted that no TDRS were produced during 120 minutes heating at 70° C., but they were produced slowly in skim milk during heating at 75° C. The maximum amounts of TDRS were produced following a brief (2 to 5 minutes) exposure at 85 and 95° C. The decline in reducing groups during long heating where the object is that of obtaining maximum amounts of reducing substances.

The amount of TDRS obtained from the heat treatment of caseinate or caseinate plus lactose is practically negligible (fig. 2B).

The influence of heat treatment on TDRS in buffered serum proteins and buffered serum proteins plus lactose is illustrated in figures 2C and 2D, respectively. These two systems behave in a similar manner in their response to heat treatment when measured as TDRS, with the exception that the presence of lactose has a rather small but significant depressing effect on the amount of TDRS produced at each temperature. An incomplete reaction between the liberated sulfhydryl groups and the lactose or an inhibition by lactose of the denaturation of the protein (16) are alternate explanations for this difference. More TDRS are produced in serum protein systems than in an equivalent of the skim milk from which they were derived. This increased heat lability may be due either to slight denaturation of the protein during isolation or to the differences of environment in the milk and the simplified systems.

TABLE 2

Comparison of the nitroprusside and thiamin disulfide methods for detection of appearance and subsequent disappearance of heat-labile sulfur in milk

Appearance			Disappearance		
Time heated at 85° C.	Nitro- prusside	TDRS (Cysteine-HCl)	Initial TDRS ^a (Cysteine-HCl)	After storage ^b	
				Nitro- prusside	TDRS (Cysteine-HCl)
(sec.)		(mg./liter)	(mg./liter)		(mg./liter)
0	nil	nil	14.4	+++	12.6
20	nil	0.2	14.4	++	3.3
30	±†	0.6	14.4	+	1.8
45	++	> 2.4	11.4	nil	nil
60	+++	> 2.4			

^a Milk was preheated at 85° C. for 20 minutes.

^b These samples were selected from among a number of comparisons. Storage was for one week at temperatures ranging from 4.4° C. to 37° C.

The data in figure 3 were obtained for comparison of AFRS and TDRS in the same systems. The capacity of milk to reduce ferricyanide recently has been considered in another paper (4). It should be pointed out that while the values of the TDRS discussed in the preceding paragraphs ranged from the equivalent of from 0.0 to 30 mg. of cysteine-HCl per liter, the AFRS on the same basis may exceed 500 mg. under similar conditions.

Only those systems containing both lactose and protein are characterized by the production of appreciable amounts of AFRS (fig. 3A, 3B and 3D) during heat treatment at 95° C. for 2 hours. The initial reducing capacity of the serum proteins is higher if measured by the acid ferricyanide method, but the two methods reveal a comparable increase in reducing capacity upon heating (compare fig. 2C and 3C).

It is difficult to prove exactly what groups in the protein are measured by thiamin disulfide. Some data have been obtained, however, which indicate that positive nitroprusside tests and the initial appearance of TDRS occur at about

the same level of heat treatment. Furthermore, upon storage of heated milk in air, the decrease in intensity and final disappearance of the reactive groups responsible for a positive nitroprusside test is paralleled by the level of TDRS. These data are shown in table 2. The nitroprusside and thiamin disulfide tests appear to detect the same groups.

Influence of oxygen tension on production of reducing groups by heat treat-

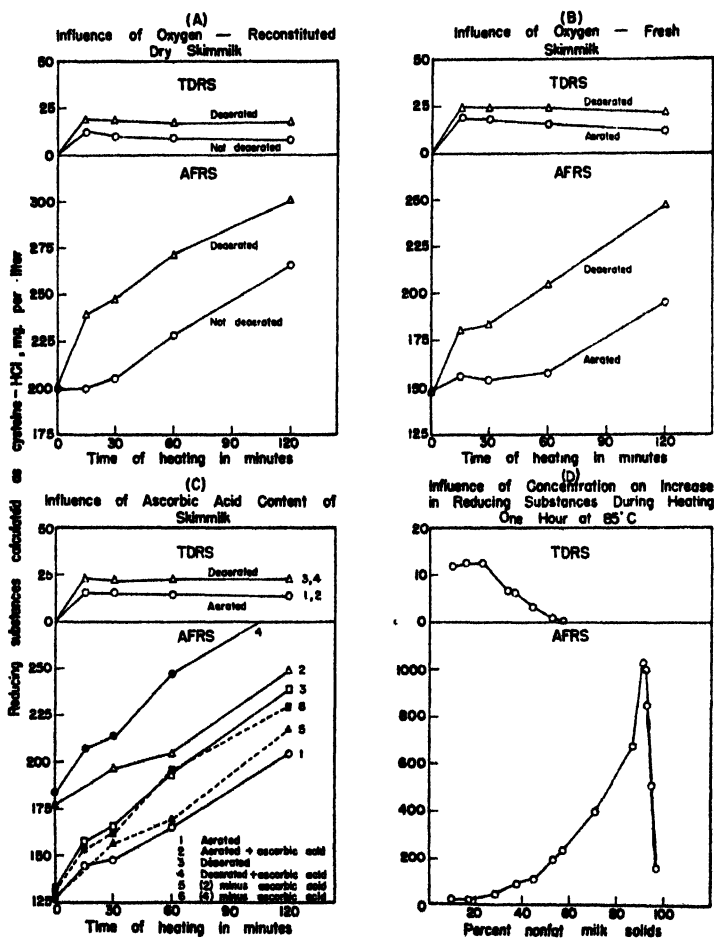


FIG. 4. The influence of oxygen, ascorbic acid and solids concentration on the reducing substances produced in nonfat milk solids during heat treatment at 85° C.

ment. As mentioned earlier, it was found in preliminary trials that the presence of oxygen in the system caused a decrease in the amount of reducing substances produced during heating. Some further work on this point is reported in figure 4. The influence of deaeration before heat treatment at 85° C. on the production of AFRS and TDRS in reconstituted nonfat dry milk solids and fresh skim milk

is shown in figures 4A and 4B, respectively. The presence of oxygen causes lower values for both AFRS and TDRS. Since the rates of production of AFRS become similar after about 30 minutes heating, regardless of the presence or absence of oxygen, it was considered possible that the initial lag in the production of reducing groups might be due to oxidation of the ascorbic acid. In an attempt to clarify this point, the data of figure 4C were obtained. The influence of oxygen and ascorbic acid on the TDRS during heating of skim milk is shown in the upper portion of figure 4C. Ascorbic acid was without effect on the TDRS, either in the presence or absence of oxygen. This result was not unexpected, since the thiamin disulfide reagent is not reduced by ascorbic acid. Any effect of ascorbic acid in this case would have to be indirect.

Curves 1 and 3 (fig. 4C) were obtained for the production of AFRS in samples of milk from which the ascorbic acid had been oxidized completely. Curve 1 represents an aerated and curve 3 a deaerated sample. Curves 2 and 4 are plots of similar data for the production of AFRS in the presence of 20 mg. of added ascorbic acid per liter. Curves 5 and 6 result from subtracting the ascorbic acid from curves 2 and 4, respectively. Since the values for AFRS shown in curves 5 and 6 are similar to the corresponding curves 1 and 3 and also since curves 2 and 4 and 1 and 3 exhibit comparable effects of the presence of oxygen, it may be concluded that ascorbic acid is not a factor in determining the amount of AFRS produced during the heating of milk.

Influence of concentration of milk solids on production of reducing substances. In the course of this work, it was discovered that the concentration of the milk solids is an important factor in the effect of heat on the production of AFRS and TDRS in milk. Nonfat dry milk solids were used to prepare systems containing from 9.7 to 96.6 per cent solids for the determination of the production of AFRS during heating (fig. 4D). The rate of production of AFRS increases very rapidly with increase in the concentration of the milk within the range of 40 to 90 per cent solids, but decreases rapidly as the system approaches the moisture content of normal dry milk. This decrease in rate of production of AFRS with a decrease in the moisture content of dry milk recently has been reported by Coulter *et al.* (3).

The systems used for studying the effect of concentration on the production of thiamin disulfide reducing substances by heat were prepared by condensing fresh skim milk in an all-glass apparatus under reduced pressure. This was found to be necessary because attempts to prepare concentrated systems by dispersing nonfat dry milk solids in water resulted in a certain amount of the proteins becoming insoluble. The data obtained for the TDRS are indicated in the upper portion of figure 4D. The TDRS produced during heating remained fairly constant up to about 23 per cent solids and fell off rapidly to zero at 57 per cent solids.

DISCUSSION

A comparison has been made of acid ferrieyanide and thiamin disulfide as oxidizing agents for heat-produced reducing substances in milk. The acid fer-

ricyanide reagent is the more powerful oxidant, and consequently reacts with other reducing groups in addition to those oxidized by the thiamin disulfide reagent. Since the nature of the AFRS has been discussed adequately in a recent paper by Crowe *et al.* (4), only comparisons with TDRS are necessary here.

Unheated milk or milk heated at temperatures up to 70° C. does not contain any substance capable of reducing thiamin disulfide, while unheated milk contains materials in addition to ascorbic acid which reduce acid ferricyanide. The TDRS are derived from heat denaturation of serum proteins. This is not surprising, since these proteins are known to contain most of the cystine-cysteine sulfur found in milk.

Although it is difficult to prove exactly what groups associated with heat treated milk serum proteins are measured by thiamin disulfide, the data presented in this paper show that this reagent probably is reduced by the same groups (undoubtedly sulfhydryls) that reduce the nitroprusside reagent used by several investigators in the past, notably Josephson and Doan (13). These workers have associated substances that reduce sodium nitroprusside with those producing a cooked flavor or those that provide the antioxygenic qualities to heated milk.

Although the presence of TDRS in dry whole milk appears to be associated with resistance to fat oxidation, these reducing substances did not decrease during storage of dry whole milk for 4 weeks in O₂ at 37° C. Even though Lea *et al.* (6, 15, 17) did not report any data concerning the stability of sulfhydryl-type reducing substances in aged dry whole milk, it is their contention that the initial presence of nitroprusside-reducing substances is concomitant with the resistance of the fat to oxidation in the stored milk.

The definitely higher reducing values obtained by heating milk in an atmosphere essentially free of oxygen agree with the statements of Greenbank and Wright (8) that deaeration of milk results in lower oxidation-reduction potential and greater protection of the resulting dry milk from oxidation. The results in figure 4C show that this effect is independent of the ascorbic acid content of the milk.

The fact that AFRS may be produced during spray drying and TDRS are not so produced can be accounted for by consideration of the effect of solids concentration on their production. TDRS are not produced on heating skim milk having a solids concentration higher than 60 per cent, whereas AFRS are produced at a maximum rate at about 90 per cent solids. Kitzes (14) has shown that the temperature of the drying particle is at about the wet-bulb temperature of the air during the early stages of drying but approaches that of the dry-bulb temperature of the air during the later stages. Thus the particle is subjected to high temperature only when its concentration is so high that TDRS are not produced.

SUMMARY AND CONCLUSIONS

Data have been presented indicating the source of thiamin disulfide reducing substances (TDRS) in heated milk and their relationship to acid ferricyanide

reducing substances (AFRS) as well as to the keeping quality of the dry whole milk. The influence of the oxygen tension and the concentration of the system on the production of TDRS and AFRS during the heat treatment have been studied.

The nitroprusside and thiamin disulfide methods for sulfhydryl groups in milk have been compared.

The following conclusions may be drawn from the results secured:

1. The substances that reduce thiamin disulfide are derived from the heat treatment of serum proteins and represent only a portion of the materials oxidized by ferricyanide.

2. The thiamin disulfide reducing substances appear to be associated with increased resistance to oxidation when present in dry whole milk but do not decrease in quantity as the milk fat is oxidized.

3. The presence of oxygen in milk being preheated depresses the amount of reducing substances produced.

4. The rate of production of thiamin disulfide reducing substances decreases with increase in the solids content of heated milk and becomes negligible at solids concentrations exceeding 60 per cent.

5. The rate of production of acid ferricyanide reducing substances in heated nonfat milk solids reaches a maximum at about 90 per cent solids.

6. The thiamin disulfide and nitroprusside reagents measure the same or parallel reducing systems in heated milk.

ACKNOWLEDGMENTS

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned no. 227 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of the Army.

REFERENCES

- (1) American Dry Milk Institute. The Grading of Dry Whole Milk and Sanitary and Quality Standards Including Standard Methods of Analysis. American Dry Milk Institute Bull. 913. 1947.
- (2) CHAPMAN, R. A., AND MCFARLANE, W. D. A Colorimetric Method for the Estimation of Reducing Groups in Milk Powders. Can. J. Research, 23B: 91-99. 1945.
- (3) COULTER, S. T., JENNESS, R., AND CROWE, L. K. Some Changes in Dry Whole Milk During Storage. J. Dairy Sci., 31: 986-1003. 1948.
- (4) CROWE, L. K., JENNESS, R., AND COULTER, S. T. The Reducing Capacity of Milk and Milk Products as Measured by a Modified Ferricyanide Method. J. Dairy Sci., 31: 595-610. 1948.
- (5) DOAN, F. J., AND JOSEPHSON, D. V. Observations on the Ascorbic Acid Content of Evaporated Milk. J. Dairy Sci., 26: 1031-1041. 1943.
- (6) FINDLAY, J. D., HIGGINBOTTOM, C., SMITH, J. A. B., AND LEA, C. H. The Effect of the Preheating Temperature on the Bacterial Count and the Storage Life of Whole-milk Powder Spray-dried by the Krause Process. J. Dairy Research, 14: 378-399. 1946.

- (7) GOULD, I. A., AND SOMMER, H. H. Effect of Heat on Milk with Especial Reference to the Cooked Flavor. Mich. Agr. Expt. Sta. Tech. Bull. 164. 1939.
- (8) GREENBANK, G. R., AND WRIGHT, P. A. The Formation and Preservation of Antioxidants by Special Methods of Processing in the Preparation of Dried Milk. J. Dairy Sci., 31: 698-699. 1948.
- (9) HARLAND, H. A., AND ASHWORTH, U. S. The Use of Thiamin Disulfide for the Estimation of Reducing Substances in Processed Milk. J. Dairy Sci., 28: 15-23. 1945.
- (10) HOLLENDER, H. A., AND TRACY, P. H. The Relation of the Use of Certain Anti-oxidants and Methods of Processing to the Keeping Quality of Powdered Whole Milk. J. Dairy Sci., 25: 249-274. 1942.
- (11) JACK, E. L., AND HENDERSON, J. L. Preventing Off-flavors in Dried Whole Milk. Food Ind., 14: 50-52. 1942.
- (12) JENNESS, R., AND COULTER, S. T. Measurement of Fluorescent Materials in Milk and Milk Products. J. Dairy Sci., 31: 367-381. 1948.
- (13) JOSEPHSON, D. V., AND DOAN, F. J. Observations on Cooked Flavor in Milk—Its Source and Significance. Milk Dealer, 29, 2: 35-36, 54-62. 1939.
- (14) KITZES, ARNOLD S. Factors Influencing the Design and Operation of a Spray Drier. Thesis, Univ. of Minn. 1948.
- (15) MATTICK, A. T. R., HISCOX, E. R., CROSSLEY, E. L., LEA, C. H., FINDLAY, J. D., SMITH, J. A. B., THOMPSON, S. Y., AND KON, S. K. The Effect of Temperature of Pre-heating, of Clarification, and Bacteriological Quality of the Raw Milk on the Keeping Properties of Whole Milk Powder Dried by the Kestner Spray Process. J. Dairy Research, 14: 116-159. 1945.
- (16) TOWNLEY, R. C., AND GOULD, I. A. A Quantitative Study of the Heat-labile Sulfides of Milk. III. Influence of pH, Added Compounds, Homogenization and Sunlight. J. Dairy Sci., 26: 853-867. 1943.
- (17) WHITE, J. C. D., SMITH, J. A. B., AND LEA, C. H. The Effect of a High Pre-heating Temperature With and Without Ethyl Gallate on the Storage Life of Whole-milk Powder Spray-dried on a Gray-Jensen Drier. J. Dairy Research 15: 127-139. 1947.

THE EFFECT OF VARYING PROPORTIONS OF EGG YOLK AND SODIUM CITRATE BUFFER IN BULL SEMEN DILUTERS UPON SPERM MOTILITY

ERIC W. SWANSON

*Department of Dairying, University of Tennessee, Agricultural
Experiment Station, Knoxville, Tennessee*

Since the first report of the influence of egg yolk upon bull sperm viability by Phillips (5), some form of egg yolk has been a major ingredient of a wide variety of diluters proposed for bull semen. Diluters containing egg yolk have produced satisfactory results with phosphate, citrate and sulfate buffers. Because of the ease of preparation and examination the citrate buffer has been the most popular in this country. Two separate types of investigation have made it of importance to examine diluters composed of egg yolk and citrate with a view to determining optimum concentrations for sperm viability.

An early report by Salisbury *et al.* (8) indicated satisfactory results with M/15 sodium citrate and egg yolk buffer, but weights listed in a later report (11) indicated that a M/7.5 solution had been prepared. The latter was used widely with apparently satisfactory results. The writer also had used M/15 citrate solutions satisfactorily. When the discrepancy was noted, a comparison was made between the freezing point depressions of fresh bull semen and the buffer solutions. As a result it was decided to use a concentration about mid-way between M/15 and M/7.5. Salisbury and Knodt (9) published a revised formula for citrate-egg yolk diluter using 3.6 per cent sodium citrate dihydrate. This revision later was explained (10) as being made to correct the osmotic pressure of the diluter to that of bull semen at the time of dilution. In the latter report Salisbury *et al.* (10) found that fresh normal bull semen had the same osmotic pressure as bovine blood and recommended a concentration of 2.9 per cent sodium citrate dihydrate to achieve this in the semen diluter prepared by heating. In view of apparently satisfactory results with such a wide variety of concentrations of sodium citrate, it seemed advisable to determine the limits of citrate concentration for satisfactory use in semen diluters.

Phillips' (6) original recommendation was for a mixture of one part of fresh egg yolk with one part of buffer solution. This pattern generally has been followed by all other investigators proposing the use of egg yolk in semen diluters. Mayer and Lasley (3, 4), in a study to determine the active fractions of egg yolk in protecting sperm against temperature-shock, found that egg yolk contained a harmful acetone-insoluble, alcohol-soluble fraction and a fraction insoluble in alcohol, acetone or ether, which was beneficial in minute amounts. Although they obtained a yield of 1.5 per cent of the latter material from egg yolk, it gave better protection than egg yolk at dilutions as high as 1 in 250 parts of buffer. These results suggested that the protective action of egg yolk might be obtained at lower concentrations than 50 per cent.

Received for publication December 2, 1948.

EXPERIMENTAL

The effect of various concentrations of sodium citrate on bull semen was determined first, since any harmful effect of the buffer could mask the true effect of egg yolk in small concentrations. At the same time the protective action of large amounts of egg yolk also might protect the sperm against adverse concentrations of sodium citrate. A few preliminary trials showed that 25 per cent egg yolk gave practically the same effect on bull semen as 50 per cent in M/15 citrate diluter. Therefore the concentration of egg yolk was decreased to this point to allow for greater effect of the sodium citrate buffer solutions. Buffer solutions were prepared by mixing at room temperature 1, 2, 3, 4 and

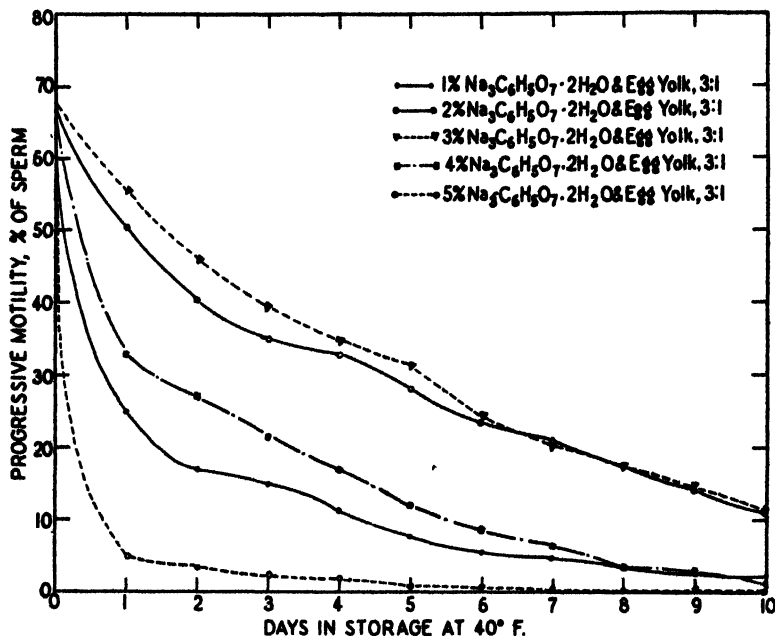


FIG. 1. Effect of various concentrations of sodium citrate in a yolk-buffer semen diluter upon motility of bull sperm (av. of 10 semen specimens).

5 g. of sodium citrate dihydrate per 100 ml. of water doubly distilled in glass. The semen diluters were prepared by mixing three parts of each buffer solution with one part of carefully separated egg yolk in 50 ml. graduated cylinders. Semen specimens from dairy bulls at the University of Tennessee were diluted identically with each of the five diluters before cooling below 28° C. The diluted samples were cooled slowly in insulated vials in a small electric refrigerator operating at 5° C. Estimations of the percentage of progressively motile sperm were made at daily intervals for 10 days. The average results from ten different semen specimens diluted at the rate of one part of semen to ten or twenty parts of each diluter are shown graphically in figure 1. It is apparent that the diluters made from 2 or 3 per cent sodium citrate dihydrate allowed better

TABLE 1

Freezing points of various sodium citrate buffer solutions and semen diluters prepared from three parts buffer and one part egg yolk

Concentration of $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$	Freezing point	
	Buffer	Diluter
(%)	(°C.)	(°C.)
1	-0.19	-0.25
2	-0.38	-0.40
3	-0.57	-0.56
4	-0.72	-0.70
5	-0.88	-0.85

motility than the others, and in comparison with these the 4 per cent citrate diluter seemed unsatisfactory. The 5 per cent citrate diluter had an immediate adverse effect on sperm motility. The 1 per cent citrate diluter was tolerated better than the 5 per cent, but, in addition to failing to maintain satisfactory motility, it caused a high proportion of coiled-tail sperm which could move only backwards or in circles.

In view of the importance of osmotic pressure upon cell metabolism, the freezing points of the buffer solutions and the diluters prepared in this experiment were determined. These data are presented in table 1. For comparison, the freezing point of a composite semen sample from three bulls was determined.

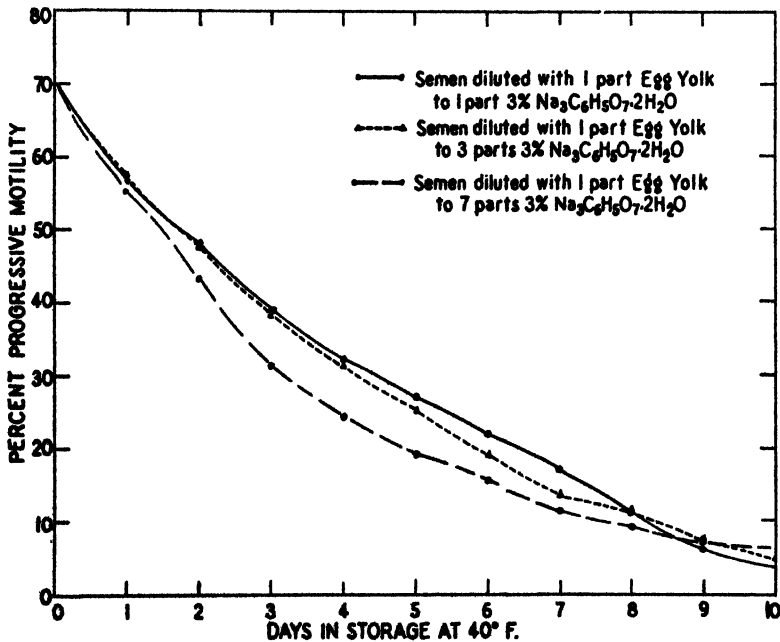


FIG. 2. Comparison of effect of 50, 25, and 12.5% egg yolk in semen diluter upon motility of bull sperm (av. of 11 semen specimens).

The freshly collected semen was cooled in ice water and the freezing point was determined within 1 hour. An average value of -0.56°C . was found. The diluter prepared with 3 per cent citrate buffer was found to have the same freezing point as fresh bull semen. This solution also resulted in the best average motility. With two of the semen samples the diluter made with 2 per cent citrate resulted in motility equal to that in the 3 per cent citrate diluter. In no instance was the 4 per cent diluter equal to the 3 per cent diluter. Apparently bovine sperm are more sensitive to hypertonic solutions of sodium citrate than to hypotonic solutions. The freezing point of four egg yolks averaged -0.57°C . It would be expected that the egg yolk would not affect significantly the tonicity of a diluter made with isotonic buffer solution.

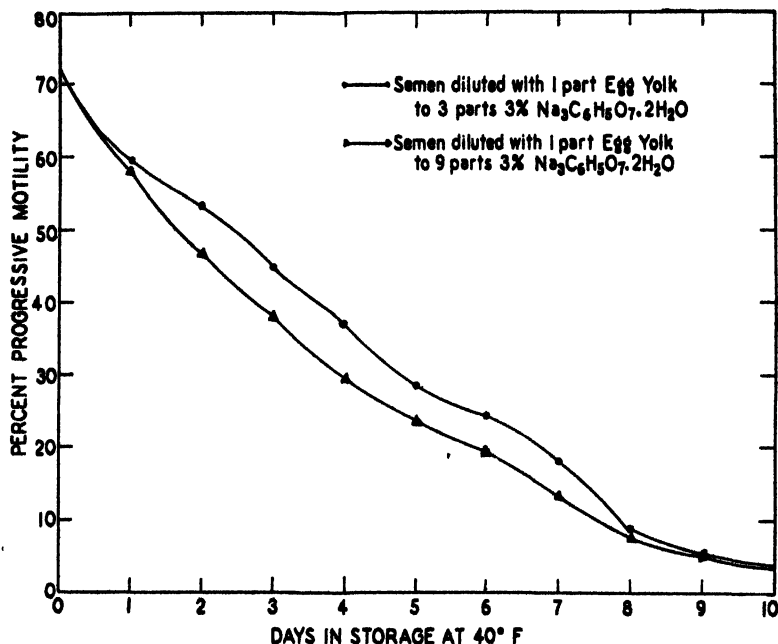


FIG. 3. Comparison of effect of 25 and 10% egg yolk in semen diluter upon motility of bull sperm (av. of 11 semen specimens).

In view of the results from the first part of the experiment a buffer solution of 3 per cent sodium citrate dihydrate was adopted for the comparisons of egg yolk concentration. All samples of semen used in this phase of the experiment were not treated with each concentration, but series of trials were made in which three or four different concentrations of egg yolk were compared. The semen generally was diluted at the rate of one part semen to ten parts diluter. In the first trial 11 semen specimens were diluted identically with diluters made with 50 per cent, 25 per cent and 12.5 per cent egg yolk, respectively. The average daily progressive motility of semen stored at 5°C . noted in this trial is shown graphically in figure 2. The 25 per cent egg yolk diluter resulted in

practically the same motility as the 50 per cent diluter. The 12.5 per cent egg yolk gave very good motility for the first 2 days of storage but was definitely inferior to the other two diluters.

In the second trial comparisons were made between 25 per cent egg yolk and 10 per cent egg yolk and citrate diluter. Charts of the average daily progressive motility ratings of 11 semen specimens used in this trial are shown in figure 3. The 10 per cent egg yolk, although it was not as good as the 25 per cent diluter, maintained surprisingly good sperm motility, quite comparable to that observed with the 12.5 per cent diluter.

A third trial was made to attempt to locate the limit of egg yolk concentration which would be effective in maintaining motility. In this trial comparisons were

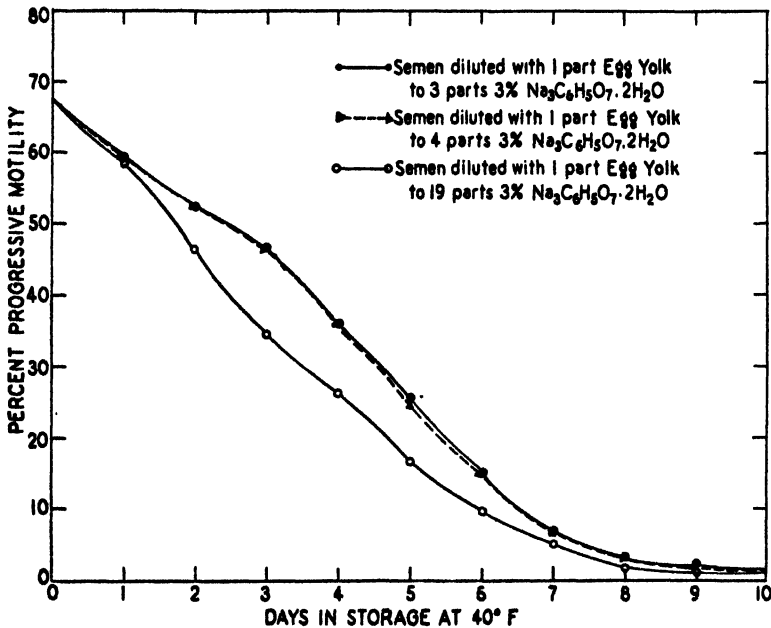


FIG. 4. Comparison of effect of 25, 20, and 5% egg yolk in semen diluter upon motility of bull sperm (av. of 11 semen specimens).

made between 25 per cent, 20 per cent, and 5 per cent egg yolk diluters. The average daily progressive motility ratings found from the 11 semen specimens used in this trial are plotted in figure 4. Identical effects were observed with 20 and 25 per cent egg yolk in the diluter. When only 5 per cent egg yolk was used, a greater difference was observed after the first day of storage than had been observed between the 10 or 12.5 per cent egg yolk and 25 per cent egg yolk diluters. It was clear, however, that even this very low concentration of egg yolk provided some beneficial effect. Two samples of semen diluted with only the buffer solution declined to 5 per cent progressive motility in 96 hours while the same semen diluted with only 5 per cent egg yolk in the diluter had 20 per cent progressive motility at 96 hours.

TABLE 2

Effect of varying proportions of egg yolk and 3 per cent sodium citrate buffer upon the resistance of bull sperm to cold shock

Diluter	Live sperm		Sperm killed
	Before shock	After shock	
	(%)	(%)	(%)
Series I (av. of 3 samples uncooled semen)			
No diluter	81	4	77
1:1, yolk:buffer	85	55	30
1:3, yolk:buffer	78	46	32
1:7, yolk:buffer	80	46	34
Series II (av. of 2 samples uncooled semen)			
No diluter	69	2	67
3% citrate buffer	74	1	73
1:3, yolk:buffer	73	35	38
1:9, yolk:buffer	74	33	41
Series III (av. of 7 samples cooled and stored semen)			
1:3, yolk:buffer	78	69	9
1:4, yolk:buffer	80	67	13
1:19, yolk: buffer	73	54	19

Since the protective action of egg yolk against temperature shock of sperm is a very important factor in the use of semen diluters, a study was made of the effect of the various concentrations of egg yolk in preventing death of sperm subjected to rapid cooling. Smears for the determination of live and dead sperm were made, by a staining technic of Lasley *et al.* (2), before and after cold shocking by placing test tubes of semen at 20° C. in a brine bath at 0 to -1° C. for 10 minutes. Three series of semen samples prepared for the storage studies reported above were treated in this manner. The cold shock treatment was given two series of samples within a few minutes after collection of the semen and before cooling had proceeded below room temperature. The third series was studied after the samples had been cooled gradually and kept in storage at 5° C. for at least 24 hours. These comparisons were presented in table 2.

TABLE 3

Effect of varying proportions of egg yolk and citrate buffer upon the resistance of bull sperm to cold shock before and after storage at 5° C.

Diluter	Before cooling			After 72 hr. at 5° C.		
	Live sperm		Sperm Killed	Live sperm		Sperm Killed
	Before Shock	After Shock		Before Shock	After Shock	
	(%)	(%)	(%)	(%)	(%)	(%)
No diluter	57	15	42	38	12	26
3% citrate buffer	65	18	47	63	33	30
1:4, yolk:citrate	71	40	31	63	50	13
1:9, yolk:citrate	70	41	29	60	48	12
1:19, yolk:citrate	68	43	25	62	43	19

It is evident from these results that diluter containing 12.5 or 10 per cent egg yolk was as effective in protecting against cold shock as was diluter containing 25 or 50 per cent egg yolk. The trial with stored semen indicated that 5 per cent egg yolk was inferior to 20 or 25 per cent in protective action, but that all concentrations exerted greater protective action than observed in uncooled semen. This effect was checked in a fourth series of three semen samples from different bulls with which the protective action of egg yolk was determined before cooling and after 72 hours storage at 5° C. The average results of this trial are presented in table 3.

These data indicate that 5 per cent egg yolk was practically as effective in protecting sperm against cold shock as were concentrations of 10 and 20 per cent. The increased resistance of sperm to cold shock after storage was noted as before, but it occurred in samples not diluted with egg yolk as well as those containing egg yolk. Some change may occur in stored sperm which affects their staining properties in addition to their acquisition of the egg yolk protective factor.

DISCUSSION

These experiments have demonstrated that the concentration of sodium citrate in the semen diluter may influence the viability of sperm. The optimum concentration was found to be that which gave a freezing point nearest that of freshly collected semen. This indicates that the influence of osmotic pressure may be important in preparing semen diluters. This is in agreement with the report of Salisbury *et al.* (10). The observation that hypotonic solutions were less harmful than hypertonic may be explained by the fact that as semen ages, lactic acid increases, causing an increase in the osmotic pressure which could be counteracted by a hypotonic solution, but only aggravates an incompatible condition in a hypertonic solution. It may be shown by later, more definitive experiments that some concentration of sodium citrate between 2.5 and 3 per cent is superior to 3 per cent.

The experiments with varying concentrations of egg yolk indicate that satisfactory results may be expected with much less than 50 per cent yolk in the diluter. The point of critical concentration was not determined definitely. From viability studies it would appear to be somewhere between 20 and 12.5 per cent. The former gave the same motility as with 50 per cent yolk, while the latter was lower in motility after the first 2 days of storage. Whether or not differences would appear in fertility cannot be decided without extensive breeding trials. Bovine semen used at the University of Tennessee has been diluted with 3 per cent sodium citrate buffer and egg yolk, three or four parts to one, routinely for over 12 months. This semen has been used for inseminating up to 3 days of age with excellent results.

Reducing the egg yolk concentration to 10 per cent had no apparent effect upon the protective action during cooling or cold shock. Since motility was not maintained as well with 10 per cent yolk as with 20 to 50 per cent, the lack of sufficient protective factor may be considered of secondary importance in mixing semen diluters with egg yolk. Factors necessary for the maintenance of the

highest motility other than protective factor must be present in egg yolk. One of these may be viscosity. Knoop (1) found that gelatin increased livability of sperm and Phillips and Spitzer (7) reported beneficial effects on sperm livability of certain gums. These substances increase the viscosity of diluted semen and reduce the motility during storage. Perhaps a certain amount of egg yolk (20 to 25 per cent) provides a similar condition.

Reducing the amount of egg yolk used in preparing semen diluter not only makes the preparation of the diluter more economical and less laborious, but results in a relatively clear field under the microscope in which estimation of semen motility is easier and more precise than when large concentrations of egg yolk are used.

SUMMARY

An investigation has been made of the egg yolk-sodium citrate buffer diluter for bull semen with the view of establishing optimum concentrations. It was found that 3 per cent sodium citrate dihydrate was superior to other concentrations tried, and this solution was nearly isotonic with fresh bull semen. Reducing egg yolk concentration to 20 per cent gave as good motility and livability as the commonly used 50 per cent. When egg yolk was reduced to 10 per cent the cold shock protective factor was still fully effective but motility was impaired slightly.

REFERENCES

- (1) KNOOP, C. E. A New Diluent for Bovine Semen. *J. Dairy Sci.*, 24: 891-892. 1941.
- (2) LASLEY, J. F., EASLEY, G. T., AND MCKENZIE, F. F. A Staining Method for the Differentiation of Live and Dead Spermatozoa. *Anat. Record*, 82: 167-174. 1942.
- (3) MAYER, D. T., AND LASLEY, J. F. The Factor in Egg Yolk Affecting Resistance and Storage Potentialities of Mammalian Spermatozoa. *J. Animal Sci.*, 3: 433. 1944.
- (4) MAYER, D. T., AND LASLEY, J. F. The Factor in Egg Yolk Affecting Resistance, Storage Potentialities, and Fertilizing Capacity of Mammalian Spermatozoa. *J. Animal Sci.*, 4: 261-269. 1945.
- (5) PHILLIPS, P. H. Preservation of Bull Semen. *J. Biol. Chem.*, 130: 415. 1939.
- (6) PHILLIPS, P. H., AND LARDY, H. A. A Yolk-Buffer Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 23: 399-404. 1940.
- (7) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (8) SALISBURY, G. W., FULLER, H. K., AND WILLETT, E. L. Preservation of Bovine Spermatozoa in Yolk-Citrate Diluent and Field Results from Its Use. *J. Dairy Sci.*, 24: 905-910. 1941.
- (9) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent Upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (10) SALISBURY, G. W., KNOTT, C. B., AND BRATTON, R. W. The Freezing Point Depression of Bull Semen and Its Relation to the Diluter Problem. *J. Animal Sci.*, 7: 283-290. 1948.
- (11) WILLETT, E. L., AND SALISBURY, G. W. The Effect of Various Diluters, Cooling Rate, Temperature of Storage, and Some Other Factors on the Livability of Spermatozoa in Stored Samples of Bull Semen. *Cornell Agr. Expt. Sta. Mem.* 249. 1942.

OXYGEN DAMAGE TO BULL SPERMATOZOA AND ITS PREVENTION BY CATALASE¹

N. L. VANDEMARK², G. W. SALISBURY², AND R. W. BRATTON

Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry, Cornell University, Ithaca, New York

In the practice of diluting and storing bull semen for use in artificial breeding it has been accepted generally that the spermatozoa remained motile longer if stored with a minimum of air space above the semen and subjected to a minimum of mixing.

MacLeod (5) showed in 1941 that high oxygen tensions were harmful to human spermatozoa. On the basis of the first experiment reported here, which was completed in 1944, and other evidence, Salisbury (10) reported that bull spermatozoa in low concentrations were harmed by oxygen.

MacLeod (6) found that adding catalase or peroxidase to human semen would prevent the harmful effects of the oxygen. This suggested that the deleterious effect of oxygen on spermatozoa was due in part to hydrogen peroxide. Hydrogen peroxide has been shown to be toxic to human (6) and bull spermatozoa (3).

Recent investigations by Tosic and Walton (15, 16) and by Tosic (14) have shown that bull spermatozoa stored under aerobic conditions produced hydrogen peroxide. They have shown that an oxidative deamination of L-tryptophane, L-tyrosine, or L-phenylalanine by the spermatozoa was responsible for the production of hydrogen peroxide and this in turn resulted in an inhibition of spermatozoan motility and respiration. This inhibition was reversed when catalase was added.

These reports suggest that oxygen damage to diluted bull semen may occur in the routine handling of bull semen for artificial insemination, and that the procedures carried out involving shaking the semen may increase these effects through increased aeration and exposure to the oxygen of the air. Since the experiments reported in this paper were completed, Prince and Almquist (9) have shown that agitation of diluted bull semen was more harmful in partially filled tubes than in full tubes.

The experiments reported here were designed to determine (a) the effect of storing diluted bull semen under nitrogen, oxygen and air on the activity of the spermatozoa, (b) whether deleterious effects from oxygen, if present, could be prevented by catalase, (c) whether oxygen damage to spermatozoa is increased by gentle mixing of diluted semen under conditions of routine artificial breeding and, (d) whether or not catalase would improve livability of spermatozoa under routine storage conditions.

Received for publication December 6, 1948.

¹ The data published in this paper have been taken from a thesis presented by the senior author to the Graduate School, Cornell University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, September, 1948.

² Present address: Department of Dairy Production, University of Illinois, Urbana, Illinois.

EXPERIMENTAL PROCEDURE

The semen samples used for these experiments were collected from the bulls of the New York Artificial Breeders' Cooperative and examined by the procedures described earlier (17). Each of the ten ejaculates used in testing the effects of incubation and storage under air, oxygen and nitrogen was diluted at the rate of one part of semen to four parts of the yolk citrate diluent (13). One ml. aliquots of the diluted semen then were measured into three series of four test tubes each. Each series consisted of one small 10 × 75 mm. tube and three larger 18 × 150 mm. tubes. The small test tube and one large test tube of each series then were stoppered; these were considered as being "under air." The other two large tubes of each series were closed with two-holed rubber stoppers equipped with one long and one short glass tube. One of these large test tubes of each series then was oxygenated for 0.5 minute by allowing a slow stream of oxygen to blow on the surface of the diluted semen while the semen was agitated gently. Following oxygenation the tube was closed immediately by means of rubber tubes and pinch clamps. A similar operation was carried out with the remaining tube of each series using nitrogen. One series of the four variously treated tubes then was incubated for 1 hour at 46.5° C.; the other two series were cooled slowly and stored at 5° C.

Aliquots of the diluted semen were taken prior to and at the end of incubation for 1 hour and after low-temperature storage of 4 and 10 days for analyses to determine the change in utilizable sugars and the accumulation of lactic acid. At the time when these analyses were carried out, the change in total reducing sugars was thought to be essentially a glucose loss; however, more recent findings by Mann (7) have shown the principal sugar in semen to be fructose. The changes in reducing sugar, called sugar loss, and lactic acid reported in this paper were obtained by difference between analyses prior to and those after incubation and after low-temperature storage. Slight modifications of the micro blood sugar method of Horvath and Knehr (2) and the lactic acid method of Barker and Summerson (1) were used for the analyses.

The percentage and rate of spermatozoan motility were estimated following both incubation and low-temperature storage.

In testing the effectiveness of catalase in preventing the harmful action of oxygen on spermatozoa, ten ejaculates of bull semen at dilution rates of one part of semen to four or 100 parts of egg yolk-citrate diluent were used (11). One ml. aliquots of each then were measured into three series of four 18 × 150 mm. test tubes. To two of the tubes of each series was added 0.1 ml. M/15 phosphate buffer containing approximately 0.2 to 0.4 of a unit of beef liver catalase; the other two tubes of each series received 0.1 ml. additions of the phosphate buffer. One tube without and one tube with added catalase of each series then were stoppered and considered as being "under air". The remaining two tubes of each series were oxygenated as described above.

One series of each dilution rate was incubated for 1 hour at 46.5° C. and the other two were cooled and stored at 5° C. At the termination of incubation and after 4 days and 10 days of low-temperature storage, the samples were opened

and the percentage and rate of spermatozoan motility estimated by microscopic examination.

To test the effects of gentle mixing on spermatozoan livability during low-temperature storage and to determine simultaneously whether catalase would improve livability, ten samples of semen diluted at 1:4 and 1:100 with the yolk-citrate diluent were used. Four 15 × 125 mm. test tubes, two with 0.2 ml. M/15 phosphate buffer added and two with 0.2 ml. of the phosphate buffer containing 0.4 to 0.8 of a unit of catalase added, were prepared for each ejaculate and each dilution rate. Two ml. of diluted semen were added to each tube, mixed and the tubes stoppered and cooled to 5° C.

During storage at 5° C., one tube with and one tube without added catalase of each dilution and each ejaculate was mixed gently at approximately hourly intervals. Each gentle mixing consisted of inverting the tubes ten times very slowly during the course of about 0.5 minute and then removing the stoppers and replacing them after another 0.5 minute. This operation was carried out on the average of seven times a day for the first 5 days and then discontinued. This was considered to duplicate to a large extent the conditions of mixing the semen at each breeding as carried out by inseminators in the field.

The remaining tubes, one with and one without added catalase at each dilution, were used as controls and were mixed only once a day at the time of examination for motility. Motility examinations were made daily for 10 days on all samples.

RESULTS

Spermatozoan activity during incubation and storage under air, oxygen and nitrogen. The mean initial semen characteristics of the ten ejaculates used in this study were as follows: sperm count, 988,000 per mm.³; initial motility, 63 per cent; rate of motility, 3.65 (maximum = 4); and methylene blue reduction time, 10.5 minutes. The average quality of these ejaculates was slightly below that normally required for actual breeding by the New York Artificial Breeders' Cooperative.

The results of this study are summarized in table 1, where the means of the observations on the ten ejaculates during incubation and storage are presented. This table is arranged so that the results for the four tubes of a series fall into the order of decreasing quantity of oxygen in the tubes (O₂, large tube with a large volume of air, small tube with comparatively less air, and N₂).

From table 1 it can be seen that, with the exception of the small tube and large tube at low-temperature storage after 4 days, each decrease in the relative amount of oxygen present resulted in a slower decline in the percentage and rate of spermatozoan motility. Although the methods used did not give an absolute measure of the oxygen present, the results show that reducing the relative amount of oxygen sealed in the storage tube produced a notable increase in livability during incubation and low-temperature storage. An analysis of variance of the motility observations showed a highly significant difference between treatments (air, O₂, and N₂) for both percentage and rate of motility.

Under oxygen, less sugar disappeared during incubation and storage for 4 days than was lost from the other treatments. However, after storage for 10 days there were no significant differences in sugar losses between any of the treatments. This was the same as was found earlier when sugar disappeared after storage for 10 days independent of semen quality (12).

Lactic acid gain, on the other hand, paralleled the increases in motility with the exception of the nitrogen treatment. In every case less lactic acid accumulated under nitrogen than under air, but more than under oxygen. Under oxygen less lactic acid was present at 10 days than was present at 4 days. This suggests that the lactic acid was oxidized.

The figures for the percentage recovery of sugar lost as lactic acid indicate that during storage for 4 days lactic acid was produced from some source other

TABLE 1

Mean per cent motility, rate of motility, sugar loss, lactic acid gain and per cent recovery of sugar lost as lactic acid in 10 ejaculates of bull semen after incubation and low temperature storage under air, oxygen, and nitrogen

After incubation for 1 hr. at 46.5° C.				After storage at 5° C.							
				4 days				10 days			
O ₂	Large tube ^a	Small tube ^a	N ₂	O ₂	Large tube ^a	Small tube ^a	N ₂	O ₂	Large tube ^a	Small tube ^a	N ₂
Motility (%)											
1	21	30	40	34	55	55	63	10	32	34	39
Rate of motility (max. = 4.0)											
0.1	1.2	1.6	2.2	1.7	3.1	3.1	3.3	0.5	1.4	1.6	1.8
Sugar loss (mg./100 ml.)											
31	51	62	60	30	58	60	47	84	89	78	88
Lactic acid gain (mg./100 ml.)											
24	45	50	49	41	53	59	48	31	65	68	52
Recovery of sugar lost as lactic acid (%)											
77	88	81	82	137	91	98	102	37	73	87	59

^a Under air.

than the sugar, since the sugar loss was not enough to account for the accumulated lactic acid. From the percentage recovery of sugar lost as lactic acid under nitrogen during storage for 10 days it appears that either lactic acid is utilized by the spermatozoa, thus reducing the total accumulation, or that some of the sugar lost is broken down to a product other than lactic acid. Lardy and Phillips (4) have indicated that spermatozoa can utilize lactic acid for the maintenance of motility.

Prevention of the ill effects of oxygen on spermatozoa by the use of catalase. The results in the study of ten ejaculates of semen stored under oxygen and under air, with and without added catalase, are presented in table 2. From these data it can be seen that oxygen again was harmful to spermatozoan motility, thus confirming the findings of the first experiment. The presence of catalase in semen diluted either at the low or high rate and incubated or stored under oxygen was effective in maintaining a higher percentage of motile spermatozoa and at a higher

TABLE 2

Mean percentage and rate of spermatozoan motility of 10 ejaculates of bull semen at two dilution rates after incubation and storage under oxygen and air with and without added catalase. (Mean initial motility 63%; rate, 3.2)

After incubation for 1 hr. at 46.5° C.				After storage at 5° C.							
				4 days				10 days			
O ₂	O ₂ + Cat.	Air	Air + Cat.	O ₂	O ₂ + Cat.	Air	Air + Cat.	O ₂	O ₂ + Cat.	Air	Air + Cat.
Motility (%)				1:4 Dilution							
4	42	41	44	14	38	40	43	8	17	8	22
Rate of motility ^a											
0.3	2.2	2.4	2.5	0.6	1.8	2.0	2.2	0.4	0.8	0.4	1.0
Motility (%)				1:100 Dilution							
1	20	14	19	7	24	30	37	1	3	3	10
Rate of motility ^a											
0.1	1.4	1.2	1.4	0.4	1.3	1.5	1.7	0.1	0.1	0.2	0.5

^a Maximum = 4.

rate of motility. The results show that those samples stored under oxygen in the presence of catalase maintained their rate and percentage of motile spermatozoa as well as, or better than, those under air during incubation and after storage for 10 days at 5° C. However, after 4 days at 5° C., motility was slightly better in the samples stored under air than under oxygen with added catalase.

Without exception the samples under air to which catalase had been added

TABLE 3

Comparison of the effects of gentle mixing versus no mixing on the mean per cent of motile sperm in 10 ejaculates of bull semen at two dilution rates stored with and without added catalase. (Mean initial motility, 63%)

Sample	Days Storage at 5° C.									
	1	2	3	4	5	6	7	8	9	10
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1:4 Dilution										
Non-mixed ^a										
Control	56	53	47	42	34	24	16	10	6	4
+ Cat.	56	53	48	46	42	36	32	24	15	12
Mixed ^b										
Control	55	50	37	31	23	16	9	6	4	2
+ Cat.	56	52	47	42	38	33	28	17	12	9
1:100 Dilution										
Non-mixed										
Control	54	45	37	26	17	8	4	4	2	1
+ Cat.	54	45	38	35	26	20	17	12	7	7
Mixed										
Control	54	41	26	18	10	5	2	2	2	1
+ Cat.	54	44	36	28	20	15	10	8	5	5

^a Non-mixed = no mixing except at the daily observations.

^b Mixed = gently mixed by inverting, this operation repeated on the average of 7 times a day for the first 5 days.

were higher in per cent and rate of spermatozoan motility than the samples under air but without the added catalase. After storage for 4 days at 5° C. the samples diluted at the rate of 1 to 100 averaged 7 per cent more motile sperm than when no catalase was added. This suggests that the addition of catalase to diluted semen used in routine breeding in the field might lengthen its useful life.

From the protective action of catalase found in these studies it seems reasonable to postulate that at least a part of the deleterious effects of high oxygen tension on spermatozoan motility is caused by increased H_2O_2 production. The increased production of H_2O_2 in turn results in the more rapid death of the spermatozoa.

The effects of gentle mixing on spermatozoan motility in diluted semen and the beneficial action of catalase. The effects of gentle mixing on the maintenance of spermatozoan motility in the presence and absence of added catalase are shown in table 3. The data on the rate of spermatozoan motility closely paralleled that for percentage motility and therefore are not presented here. Gentle shaking reduced considerably the percentage of spermatozoan motility in the control samples at both the 1:4 and the 1:100 dilution rates. However, if the mixed samples contained added catalase, motility was maintained better than in non-mixed samples without added catalase. In fact, the presence of added catalase slowed the decline in the percentage of motile spermatozoa in both the mixed and non-mixed samples. Livability was greatest in the non-mixed samples with added catalase. Next were the mixed samples with added catalase, followed by the non-mixed samples without added catalase. The maintenance of motility was poorest in the mixed samples without added catalase. This latter group was nearest to the conditions normally carried out in the field in routine artificial breeding.

Since catalase was effective in preventing the ill-effects of gentle shaking, it seems reasonable to conclude that the effect of shaking was due primarily to the increased production of H_2O_2 by the spermatozoa, and not to mechanical injury. Mercier (8) found that increased gentle shaking did not increase the number of tailless heads or abnormal forms of spermatozoa found in slides prepared for staining.

The beneficial action of catalase, when samples received minimum mixing necessitated by the daily examinations, indicates that the spermatozoa were producing H_2O_2 even when subjected to little extra aeration.

DISCUSSION

Although the oxygen concentrations were not controlled carefully, the results of the first experiment indicated that near-anaerobic conditions were desirable for the maintenance of bovine spermatozoan motility. This is similar to the findings of MacLeod (5), who has shown that the higher the oxygen tension the quicker human spermatozoa lose their motility. Thus for artificial breeding purposes, diluted bull semen should be stored in test tubes with a minimum of air space left above the semen. Storage tubes of various sizes should be used to ship the semen in order to have each tube as nearly full as possible in order to cut down on the volume of air and minimize the deleterious effects due to oxygen.

Since the present-day methods of shipping and handling bull semen for use in artificial insemination involve unavoidable gentle-mixing procedures, the results of the experiments presented here suggest that adding catalase to semen diluted with the egg yolk-citrate would prove beneficial. The production of hydrogen peroxide undoubtedly is increased by mixing procedures. Added catalase would aid in the rapid destruction of the increased quantities of hydrogen peroxide produced and thereby maintain a less toxic environment for the spermatozoa, thus promoting longer livability. An experiment to test the effects of added catalase on conception results in routine artificial breeding in the field is underway and will be reported at a later date.

SUMMARY

1. Bovine spermatozoan activity was studied in the egg yolk-citrate diluent during incubation at 46.5° C. and storage at 5° C. under air, oxygen and nitrogen, under air and oxygen with and without added catalase, and during storage when subjected to minimum and routine mixing procedures with and without added catalase.

2. Oxygen produced deleterious effects which were manifested by decreased motility and livability of the spermatozoa and a reduction in their ability to convert sugar to lactic acid.

3. Oxygen damage to spermatozoa was largely eliminated when catalase was added to the diluted semen.

4. Gentle mixing shortened the life of stored bull spermatozoa in the yolk-citrate diluent, but the presence of added catalase in mixed samples obliterated the harmful effects of mixing.

5. The effectiveness of catalase in preventing the harmful action of oxygen and shaking on bull spermatozoa in the egg yolk-citrate diluent led to the conclusion that increased aeration and higher oxygen tensions speed the production of hydrogen peroxide by the spermatozoa and this in turn produces a toxic environment, shortening the life of the spermatozoa.

6. The results suggest that a minimum of air space be left above the diluted semen that is stored and shipped in routine artificial breeding and that semen samples should be subjected to a minimum of mixing.

ACKNOWLEDGMENTS

The authors are indebted to Professors J. B. Sumner and W. L. Nelson of the Department of Biochemistry for the sample of catalase used to initiate these studies and for their suggestions which were so helpful in the preparation of beef liver catalase.

REFERENCES

- (1) BARKER, S. B., AND SUMMERSON, W. H. The Colorimetric Determination of Lactic Acid in Biological Material. *J. Biol. Chem.*, 138: 535-554. 1941.
- (2) HORVATH, S. M., AND KNEHR, C. A. Adaptation of the Folin-Malmros Micro Blood Sugar Method to the Photoelectric Colorimeter. *J. Biol. Chem.*, 140: 869-877. 1941.
- (3) LARDY, H. A., AND PHILLIPS, P. H. The Effect of Certain Inhibitors and Activators on Sperm Metabolism. *J. Biol. Chem.*, 138: 195-202. 1941.

- (4) LARDY, H. A., AND PHILLIPS, P. H. Studies of Fat and Carbohydrate Oxidation in Mammalian Spermatozoa. *Arch. Biochem.*, 6: 53-61. 1945.
- (5) MACLEOD, JOHN. The Metabolism of Human Spermatozoa. Thesis, Cornell University. 1941.
- (6) MACLEOD, JOHN. The Role of Oxygen in the Metabolism and Motility of Human Spermatozoa. *Am. J. Physiol.*, 138: 512-518. 1943.
- (7) MANN, T. Studies on the Metabolism of Semen. 3. Fructose as a Normal Constituent of Seminal Plasma. Site of Formation and Function of Fructose in Semen. *Biochem. J.*, 40: 481-491. 1946.
- (8) MERCIER, ERNEST. The Effects of Season on Spermatogenic Activity and Reproduction in Cattle. Thesis, Cornell University. 1946.
- (9) PRINCE, P. W., AND ALMQUIST, J. O. The Effect of Agitation upon the Livability of Bovine Spermatozoa. *J. Dairy Sci.*, 31: 839-844. 1948.
- (10) SALISBURY, G. W. The Glycolysis, Livability, and Fertility of Bovine Spermatozoa as Influenced by Their Concentration. *The Problem of Fertility*. Edited by Earl T. Engle. Princeton University Press, Princeton, New Jersey. Pp. 134-152. 1946.
- (11) SALISBURY, G. W., KNOTT, C. B., AND BRATTON, R. W. The Freezing Point Depression of Bull Semen and its Relation to the Diluter Problem. *J. Animal Sci.*, 7: 283-290. 1948.
- (12) SALISBURY, G. W., AND VANDEMARK, N. L. Stimulation of Livability and Glycolysis by Additions of Glucose to the Egg Yolk-citrate Diluent for Ejaculated Bovine Semen. *Am. J. Physiol.*, 143: 692-697. 1945.
- (13) SALISBURY, G. W., ZELAYA, J. A., AND VANDEMARK, N. L. Livability and Glycolysis of Bovine Spermatozoa in Yolk-citrate, Incubated Eggs or Chick Embryo Diluters. *J. Animal Sci.*, 4: 270-276. 1945.
- (14) TOSIC, J. Mechanism of Hydrogen Peroxide Formation by Spermatozoa and the Role of Amino Acids in Sperm Motility. *Nature*, 159: 544. 1947.
- (15) TOSIC, J., AND WALTON, A. Respiration of Spermatozoa in Egg-yolk Medium. *Nature*, 156: 507-508. 1945.
- (16) TOSIC, J., AND WALTON, A. Formation of Hydrogen Peroxide by Spermatozoa and its Inhibitory Effect on Respiration. *Nature*, 158: 485. 1946.
- (17) WILLETT, E. L., AND SALISBURY, G. W. The Effect of Various Diluters, Cooling Rate, Temperature of Storage, and Some Other Factors on the Livability of Spermatozoa in Stored Samples of Bull Semen. N. Y. (Cornell) Agr. Expt. Sta. Memoir 249. 1942.

USE OF DEHYDRATED BANANA MEAL IN THE RATIONS OF DAIRY CALVES^{1, 2, 3}

W. D. FYOCK AND C. B. KNODT

Pennsylvania Agricultural Experiment Station

Dehydrated banana meal, because of its high pectin content, palatability and the proven value of bananas in the treatment of diarrhea in infant and adult humans (1, 2, 3, 4, 5) appeared to be of possible value in the rations of dairy calves. Analysis of dehydrated banana meal prepared from whole bananas indicated the following composition: crude protein, 4.70 per cent; ether extract, 3.10 per cent; nitrogen free extract, 76.10 per cent; crude fiber, 4.30 per cent; pectins and tannins, 9.15 per cent. A review of available literature revealed no reports relative to the use of this product in animal feeds.

The objective of the trials presented in this report was to determine the value of dehydrated banana meal for growth and for the prevention of scours in dairy calves.

EXPERIMENTAL PROCEDURE

Six groups of 10 Holstein male calves each were used in the trials presented in this report. The calves were obtained in eastern Pennsylvania from private and state-owned herds. At the beginning of the trials, the calves were divided into six comparable groups on the basis of body weight, height at withers, circumference of the chest, and age.

The calves were housed in an artificially heated and ventilated barn. They were maintained in individual pens provided with a water bowl, grain box, hay rack and salt block. The calves were distributed throughout the barn to avoid as much as possible any positional effects. Measurements of body weight, height at withers and chest circumference were taken each Tuesday at 9:00 a.m. and the calves always were weighed in the same order. Daily observations were made of the condition of the feces of each calf. All observations were made by the same person to minimize as much as possible any variation. Six categories were set up relative to the general character of the feces as follows: W = white scours; WB = white scours with blood; N = normal; N1 = loose; N2 = loose +; N3 = very loose; N4 = watery.

All calves were given one bolus (4 g.) of sulfathalidine at the time of each of the first four feedings after arrival at the experimental barn. If scours oc-

Received for publication December 9, 1948.

¹ Taken from data presented in a thesis to the graduate faculty of the Pennsylvania State College by W. D. Fyock in partial fulfillment of the requirements for the degree of Master of Science.

² The authors wish to express their appreciation to the United Fruit Company, New York, N. Y., for a research grant which assisted in making this study possible, and to Sharp and Dohme, Inc., Glenolden, Pennsylvania, for supplying the sulfathalidine used in this trial.

³ Authorized for publication on November 19, 1948, as paper No. 1489 in the journal series of the Pennsylvania Agricultural Experiment Station.

curred and lasted more than 24 hours during the trial, the calf again received the above treatment.

The calves were fed whole milk, which averaged about 3.7 per cent butterfat, from the College research herd. This was fed twice a day according to the following schedule:

1-4 weeks	10 lb. per day
5 "	8 " " "
6 "	6 " " "
7 "	4 " " "

In addition to milk, the calves were fed their respective group grain mixture *ad libitum* up to 6 lb. per day. All of the grain mixtures as presented in table 1 contained approximately 17.35 per cent of digestible protein. Group I served

TABLE 1
Rations used

Ingredients	Group I	Group II	Group III	Group IV
			(lb.)	
Banana meal	0	100	400	800
Ground yellow corn	600	500	200	125
Rollod oats	500	488	450	50
Wheat bran	200	200	200	200
Soybean meal	250	262	300	375
Dried skim milk*	400	400	400	400
Salt	20	20	20	20
Ground limestone	10	10	10	10
Steamed bonemeal	10	10	10	10
Vit. A oil (3300 USP units/g.)	8	8	8	8
Irradiated yeast	2	2	2	2
Estimated dig. protein (%)	17.33	17.30	17.38	17.43

* At the end of 4 months 300 lb. of soybean meal were substituted for 300 lb. of skim milk powder.

as a control group and received no banana meal. Group II received a ration containing 5 per cent banana meal; group III, 20 per cent; and group IV, 40 per cent. Groups V and VI received the basal ration plus 2 and 4 ounces of banana meal, respectively, twice daily, mixed with the milk fed. The whole milk was fed to these groups at the same rate as to the other four groups.

During the first 4 weeks, orchard grass-alfalfa hay of good quality was fed *ad libitum*. After the first 4 weeks, good quality alfalfa hay was fed *ad libitum*. The calves in groups I, II, III and IV were on the trial 24 weeks. The calves in groups V and VI were on trial for 8 weeks, 1 week in addition to the milk feeding period.

RESULTS

Growth. The relative rates of growth of the various groups of calves were recorded in terms of pounds of body weight, inches of chest circumference and height at withers in inches. The summarized data at 8 and 24 weeks are presented in table 2. The most rapid growth during the first 8 weeks was made by

TABLE 2
Rates of growth of calves

	Group I	Group II	Group III	Group IV	Group V	Group VI
<i>Body weight (lb.)</i>						
Initial	102.0	100.7	99.7	99.0	103.2	111.9
8 weeks	174.3	175.6	168.9	181.4	155.4	186.6
24 weeks ..	432.6	445.6	420.8	428.6
<i>Height at withers (in.)</i>						
Initial	29.7	29.5	29.8	30.0	29.9	29.8
8 weeks	33.0	33.0	33.0	33.7	32.6	33.5
24 weeks ...	41.7	41.2	41.2	41.2
<i>Chest circumference (in.)</i>						
Initial ...	31.7	31.5	32.0	31.5	31.2	33.3
8 weeks ...	37.6	37.8	37.1	37.9	36.4	38.2
24 weeks ..	51.3	51.6	51.0	51.8

* Each datum represents a mean of the observations on all calves in the given group at the given time.

group IV. This group gained an average of 1.47 lb. daily as compared to 1.29, 1.34, 1.24, 0.93 and 1.33 lb. for groups I, II, III, V and VI, respectively. The lowest rate of growth, as indicated by body weight gain, was in group V, which was the group that received 2 ounces of banana meal mixed with each of two daily milk feedings. Statistical treatment of the data by analysis of variance indicated that there was no significant difference among groups in body

TABLE 3
Incidence of abnormal feces and use of sulfathalidine treatments during first 8 weeks (10 calves per group)

	Group I	Group II	Group III	Group IV	Group V	Group VI
<i>No. of cases^{a,b}</i>						
W	0	3	2	1	8	5
WB	0	0	0	0	0	3
N1	14	15	20	22	12	8
N2	2	3	5	2	4	7
N3	0	0	2	1	0	3
N4	4	0	2	2	0	5
Total	20	21	31	27	24	31
<i>No. calves having abnormal feces^a</i>						
Av. duration (days)	1.5	1.4	1.7	1.1	1.1	2.7
<i>No. receiving one treatment sulfathalidine^a</i>						
<i>No. receiving 3 or more sulfathalidine treatments^a</i>						
	1	4	2	2	5	4
	1	0	4	1	2	1

^a After initial treatment.

^b W = white, scours

WB = white scours with blood

N1 = loose

N2 = loose +

N3 = very loose

N4 = watery

weight after being on the trial 8 weeks. There was little variation in height at withers of the six groups at 8 weeks, the average daily gain being 0.06 inches for each group. The average daily gain in chest circumference at 9 weeks was 0.11, 0.11, 0.09, 0.11, 0.09 and 0.07 inches for groups I through VI, respectively. The differences were not great enough to be significant statistically at 8 weeks.

Measurements of body weight obtained at 24 weeks showed greater variation than did the other measurements. The average daily gains at this period were 1.97, 2.05, 1.91 and 1.96 lb. for groups I through IV, respectively. All four groups were well above the standards reported by Ragsdale (6) for body weight at 24 weeks. Analysis of variance indicated no significant differences among the four groups in body weight during the trial. Very slight differences were observed among groups in measurements of height at withers at 24 weeks. Average daily gains at this period were 0.071 inches for group I and 0.067 inches

TABLE 4
Incidence of Scours During 24-Week Trial (10 calves per group)

	Group I	Group II	Group III	Group IV
No. of cases ^a				
W	0	4	3	1
WB	0	0	0	0
N1	24	22	38	31
N2	1	4	6	6
N3	0	0	1	1
N4	0	0	2	3
Total	25	30	50	42
Av. duration of scours (days)	1.60	0.93	1.30	1.33
No. calves having scours	8	9	9	9
No. calves that received only one sulfa-thalidine treatment	1	4	2	1
No. calves receiving 2 or more sulfa-thalidine treatments	1	0	4	2

^a See table 3.

for groups II, III and IV; the differences were not significant statistically. Growth in height at withers when compared to the standards reported by Ragsdale (6) was well above normal at the end of the 24-week feeding trial. The average daily gains in chest circumference after the calves were on the feeding trial for 24 weeks were 0.12, 0.12, 0.11 and 0.12 inches for groups I through IV, respectively, and there were no statistically significant differences. All groups were above normal when compared to the standards reported by Ragsdale (6) relative to chest circumference after 24 weeks on the feeding trial.

Condition of feces. Data relative to the cases of abnormal feces are summarized at 8 and 24 weeks in tables 3 and 4, respectively. Under the conditions of this experiment there appears to be no relation between the amount of banana meal in the ration of dairy calves and the condition of the feces.

Using the average duration of abnormal feces as a criterion, there appears to be some value in the use of banana meal in the control of this condition. While

it did not decrease the incidence, it did decrease the severity at both 8- and 24-week periods, as indicated by the average duration of cases.

Feed. Indications are that dehydrated banana meal in the grain ration increased the palatability of the rations used in these trials (table 5). While the total grain consumption during the 24 weeks of the trial shows little variation between groups, there was considerable variation among amounts of grain consumed by each group during the first 6 weeks. These figures indicate that during the period when grain feeding was primarily *ad libitum*, the palatability of the grain mix was in direct proportion to the amount of banana meal in the grain mixture.

The total hay consumption during the first 6 weeks showed a tendency for the amount of hay consumed to decrease as the amount of grain mixtures consumed increased during this period.

Little variation was found among groups in the amount of grain consumed per lb. of gain. Group I required 25.0 lb. of grain per lb. as compared to 21.3,

TABLE 5
Grain and Hay Consumption

	Group I	Group II	Group III	Group IV	Group V	Group VI
	(lb.)					
Grain first 6 weeks	375	390	423	586	422	384
Total grain	7105	7349	7124	7209	712	835
Hay first 6 weeks	257	268	237	189	144	207
Total hay	8279	7754	6855	7622	470	388

22.2 and 21.9 for groups II, III and IV, respectively. Hay consumption per lb. of gain was 25.0, 22.5, 21.3 and 23.1 lb. for groups I through IV, respectively.

CONCLUSIONS AND SUMMARY

1. A study has been conducted to determine the value of dehydrated banana meal in the rations of Holstein bull calves. Grain rations containing 5, 20 and 40 per cent of banana meal were fed for a period of 24 weeks. The calves in two additional groups each received 2 and 4 ounces of dehydrated banana meal twice per day mixed with the regular milk feeding during the 7-week period of milk feeding.

2. The inclusion of dehydrated banana meal in grain rations at the levels used or in the milk fed did not increase significantly the rate of growth in terms of body weight, chest circumference or height at withers during the trial. Similarly, the feeding of 2 and 4 ounces of dehydrated banana meal with each of the twice-per-day milk feedings did not affect significantly the rate of growth during the 7-week milk-feeding period.

3. Addition of 5, 20 and 40 per cent dehydrated banana meal to the grain ration or the mixture of 2 and 4 ounces of banana meal with each milk feeding did not reduce significantly the incidence of scours under the conditions of these trials.

4. The dehydrated banana meal did improve the palatability of the rations used. Under the conditions of these trials, banana meal could be used to replace part of the ground yellow corn and rolled oats in the rations of dairy calves.

REFERENCES

- (1) BOGERT, L. J. Dietary Uses of the Banana in Health and Disease. Published by the United Fruit Co., New York City. 1942.
- (2) BRUBAKER, E. W. The Treatment of Acute Diarrheal Disorders of Infancy and Early Childhood and Bananas and Banana Powder. *J. Michigan State Med. Soc.*, **36**: 40-46. 1937.
- (3) HUNSCHER, H. A., HUMMEL, F. C., AND MACY, I. G. Effect of Varied Banana Intake on Nitrogen and Mineral Balances of Normal Children. *Am. J. Diseases Children*, **60**: 509-517. 1940.
- (4) KANTOR, J. L. The Medical Service at the First Army Maneuvers Of 1940—with Special Reference to the Work of the First Evacuation Hospital. *Military Surgeon*, **88**: 459-473. 1941.
- (5) MACY, I. G., HUMMEL, F. C., HUNSCHER, H. A., SHEPPARD, M. L., AND SAUDERS, H. J. Effects of Simple Dietary Alterations upon Retention of Positive and Negative Minerals by Children. *J. Nutrition*, **19**: 461-476. 1940.
- (6) RAGSDALE, A. C. Growth Standards for Dairy Cattle. *Missouri Agr. Expt. Sta. Bull.* **336**. 1934.

THE EFFECT OF PREPARTUM VITAMIN A SUPPLEMENTATION ON THE HEALTH AND PERFORMANCE OF THE YOUNG CALF

A. A. SPIELMAN,¹ H. D. EATON,¹ J. K. LOOSLI AND K. L. TURK
Department of Animal Husbandry, Cornell University, Ithaca, New York

The relationship of the quantitative and qualitative factors of the maternal diet other than extreme deficiencies to the health of the newborn calf has received only limited attention, yet it is apparent that not only is such information of physiological significance but also of economic importance to the dairyman.

Spielman *et al.* (21, 22) and Wise *et al.* (25) were able to demonstrate an increased amount of vitamin A in the blood and livers of newborn calves and in colostrum when the dams were fed supplementary vitamin A during the later stages of the gestation period.

The purpose of the present study was to determine the effect of prepartum vitamin A supplementation on the health and performance of the young calf.

EXPERIMENTAL PROCEDURE

A total of 59 cows of the Brown Swiss, Guernsey, Holstein and Jersey breeds calving in the Cornell University herd from October, 1946, to July, 1947, were allotted to one of four dietary groups 30 days prior to the calculated parturition date. Breed, age, number of previous gestations, length of dry period and previous feeding history were considered in placing cows in a particular dietary grouping. The four dietary groupings were as follows: (a) a basal ration of good quality timothy and clover mixed hay, corn silage and a grain mixture containing 12 per cent crude protein; (b) basal ration plus 1 million I.U. of vitamin A daily in the form of alfalfa leaf meal; (c) basal ration plus 1 million I.U. of vitamin A daily as vitamin A alcohol; (d) basal ration plus 1 million I.U. of vitamin A daily as vitamin A ester. The hay and silage contained an average of 11.8 and 5.0 γ of carotene per g., respectively, as determined by the method of Moore and Ely (10) as modified by Nelson *et al.* (11). Cows calving during October and November received a limited amount of fall pasture. These cows were divided equally among dietary groupings. The dehydrated alfalfa leaf meal contained an average of 114 mg. of carotene² per lb. It was hand mixed with the grain and fed once daily. The alcohol and ester forms of vitamin A^{2,3} were administered orally once daily in capsules. The potencies of the alcohol and ester forms of vitamin A, as measured by the Evelyn photoelectric colorimeter, were on the average 13.75 and 13.65 mg. of vitamin A per g. of oil.

Received for publication December 13, 1948.

¹ Now at the University of Connecticut, Storrs.

² It was assumed that 0.25 γ of vitamin A was equivalent to 1 I.U. of vitamin A and that 0.6 γ of carotene was equivalent to 1 I.U. of vitamin A.

³ The alcohol form of vitamin A was diluted in corn oil and was manufactured to contain 55,000 I.U. of vitamin A per g. The ester form of vitamin A was made up of the ester form of fish liver oil and manufactured to contain 55,000 I.U. of vitamin A per g. Both vitamin A supplements were supplied by Dr. André E. Briod of the Nopco Chemical Company, Harrison, New Jersey.

Calves were left with their dams for the first 2 days after birth. They then were moved to individual pens and raised according to the Cornell dry-calf starter method (23). Milk was fed twice daily from nipple pails. Each calf received its mother's milk up to 8 days of age and thereafter herd milk. Starter and mixed timothy and clover hay were fed free choice, and water was before the calves at all times. Feed intakes and refusals were recorded. Calves were weighed at birth and at weekly intervals thereafter up to 4 weeks of age. Blood samples were drawn from the jugular vein at birth and at weekly intervals thereafter up to 4 weeks of age. Plasma carotene and vitamin A were determined by the method of Kimble (8).

TABLE 1

*The effect of the prepartum diet on the carotene content of the plasma of young calves**

Breed	No. of calves	Carotene content of plasma (γ /100 ml.) at ages of:				
		Birth	1 week	2 weeks	3 weeks	4 weeks
<i>Controls</i>						
Holstein	14	2.5 \pm 0.4	11.9 \pm 1.9	10.5 \pm 1.6	12.5 \pm 2.0	17.9 \pm 3.5
Brown Swiss	2	1.5 \pm 1.5	19.5 \pm 1.5	14.5 \pm 0.5	11.5 \pm 5.5	25.5 \pm 1.5
Guernsey	2	2.0 \pm 2.0	29.3 \pm 8.3	25.8 \pm 6.3	35.5 \pm 14.5	28.5 \pm 10.5
Jersey	1	1.0	30.0	38.0	30.0	39.0
All Breeds	19	2.3 \pm 0.4	15.7 \pm 2.2	14.2 \pm 2.2	15.8 \pm 2.6	21.1 \pm 3.0
<i>Alfalfa leaf meal</i>						
Holstein	9	2.8 \pm 0.8	15.1 \pm 3.3	14.4 \pm 3.1	15.1 \pm 2.4	13.8 \pm 1.9
Brown Swiss	2	3.0 \pm 0.0	16.0 \pm 5.0	14.5 \pm 5.5	11.5 \pm 2.5	23.5 \pm 0.5
Guernsey	2	3.5 \pm 1.5	56.0 \pm 3.0	27.0 \pm 3.0	35.5 \pm 2.5	39.0 \pm 0.0
Jersey	1	5.0	20.0	14.0	21.0	30.0
All Breeds	14	3.1 \pm 0.5	21.9 \pm 4.6	16.2 \pm 2.4	17.9 \pm 2.6	20.0 \pm 2.8
<i>Alcohol form of vitamin A</i>						
Holstein	5	2.6 \pm 0.8	16.2 \pm 3.6	17.0 \pm 2.7	21.8 \pm 4.0	21.0 \pm 2.6
Brown Swiss	1	2.0	30.0	24.0	54.0	35.0
Guernsey	2	5.0 \pm 3.0	39.0 \pm 21.0	35.0 \pm 21.0	44.0 \pm 27.0	32.0 \pm 24.0
Jersey	1	3.0	23.0	14.0	26.0	20.0
All Breeds	9	3.1 \pm 0.8	23.6 \pm 5.2	21.4 \pm 4.7	30.8 \pm 6.6	24.9 \pm 4.7
<i>Ester form of vitamin A</i>						
Holstein	11	2.7 \pm 0.6	13.4 \pm 1.5	14.8 \pm 2.7	17.1 \pm 3.2	17.5 \pm 2.7
Brown Swiss	2	1.0 \pm 1.0	10.0 \pm 2.0	10.5 \pm 1.5	17.0 \pm 6.0	21.5 \pm 8.5
Guernsey	1	6.0	27.0	27.0	35.0	32.0
Jersey	2	3.0 \pm 1.0	41.5 \pm 3.5	47.0 \pm 3.0	52.0 \pm 1.0	37.0 \pm 7.0
All Breeds	16	2.8 \pm 5.0	17.3 \pm 2.8	19.1 \pm 3.4	22.6 \pm 3.8	21.3 \pm 2.7

* Values given are mean \pm standard error of the mean.

The data were treated statistically by analysis of variance as described by Love (9). Only Holstein calves were included in the reported analyses of the data because of the unequal distribution between grouping of calves of the other breeds. However, analysis including all breeds gave essentially the same differences as when only Holstein calves were included. Only those differences significant at the 1 per cent level are included as real differences. In the case of scours, the total number of days each calf was free from scours was divided by the total number of experimental days and the resulting quotient multiplied by 100 to give a percentage. The percentage then was converted to the appropri-

ate angle from Bliss's tables which are contained in Snedecor (17). The angles thus obtained then were used in analysis of variance to test for significance.

RESULTS

The levels of carotene in the blood plasma of the calves are listed in table 1. Holstein calves from dams fed the alcohol form of vitamin A were significantly higher in plasma carotene on the basis of all of the experimental data than calves from dams in the other three dietary groupings. Also, those calves from dams fed the ester form of vitamin A were higher in plasma carotene than those calves from dams fed alfalfa leaf meal. At any one age no significant differences were

TABLE 2

The effect of the prepartum diet on the vitamin A content of the plasma of young calves

Breed	No. of calves	Vitamin A content of plasma (γ /100 ml.) at ages of:				
		Birth	1 week	2 weeks	3 weeks	4 weeks
<i>Controls</i>						
Holstein	14	3.6 \pm 0.5	12.5 \pm 1.9	12.8 \pm 1.5	10.0 \pm 0.9	9.9 \pm 6.6
Brown Swiss	2	3.0 \pm 0.9	15.7 \pm 0.2	11.8 \pm 0.8	11.8 \pm 3.2	13.3 \pm 1.7
Guernsey	2	2.7 \pm 0.4	19.6 \pm 4.7	11.8 \pm 5.0	10.4 \pm 1.1	9.0 \pm 2.7
Jersey	1	5.3	11.9	11.6	6.9	7.8
All Breeds	19	3.5 \pm 0.4	13.6 \pm 1.5	12.5 \pm 1.1	10.0 \pm 0.7	10.1 \pm 0.6
<i>Alfalfa leaf meal</i>						
Holstein	9	4.5 \pm 0.7	11.1 \pm 1.8	10.3 \pm 1.2	10.2 \pm 1.0	9.0 \pm 0.8
Brown Swiss	1	1.9 \pm 0.9	11.2 \pm 0.8	7.3 \pm 0.4	10.3 \pm 0.4	10.9 \pm 1.4
Guernsey	2	0.3 \pm 0.3	8.1 \pm 3.7	9.3 \pm 4.5	8.5 \pm 0.2	9.0 \pm 4.6
Jersey	1	2.0	12.2	8.1	9.0	4.7
All Breeds	9	3.3 \pm 0.7	10.7 \pm 1.2	9.6 \pm 1.0	9.9 \pm 0.6	8.9 \pm 0.8
<i>Alcohol form of vitamin A</i>						
Holstein	5	7.2 \pm 0.8	16.3 \pm 2.7	12.6 \pm 2.0	12.8 \pm 1.9	13.7 \pm 3.1
Brown Swiss	2	8.5	17.6	15.1	16.5	11.3
Guernsey	2	7.9 \pm 0.5	13.1 \pm 5.3	10.8 \pm 2.2	14.1 \pm 4.8	11.1 \pm 1.2
Jersey	1	5.6	26.3	6.6	11.0	11.9
All Breeds	14	7.3 \pm 0.5	16.8 \pm 2.1	11.8 \pm 1.4	13.3 \pm 1.4	12.6 \pm 1.2
<i>Ester form of vitamin A</i>						
Holstein	11	9.9 \pm 0.6	17.1 \pm 1.9	14.2 \pm 1.1	13.7 \pm 1.0	13.1 \pm 0.7
Brown Swiss	2	5.9 \pm 1.1	12.1 \pm 1.1	13.8 \pm 3.1	10.8 \pm 0.8	10.0 \pm 0.1
Guernsey	1	3.3	11.6	11.6	9.1	8.4
Jersey	2	8.4 \pm 5.7	22.8 \pm 5.7	13.7 \pm 0.6	13.2 \pm 2.7	19.6 \pm 3.8
All Breeds	16	8.8 \pm 1.6	16.8 \pm 1.6	13.9 \pm 0.8	12.9 \pm 0.8	13.2 \pm 0.7

found between groupings. Calves within a grouping varied significantly among themselves during the experimental period and with age (table 1).

Vitamin A levels in the plasma of calves are given in table 2. Calves from dams fed either form of vitamin A were significantly higher in the level of plasma vitamin A based on all the experimental data than calves from control dams or from dams fed alfalfa leaf meal. There were no real differences between either group of calves from dams receiving supplemental vitamin A or between calves from control dams and dams fed alfalfa leaf meal. Significant differences existed at birth and at 3 and 4 weeks of age but not at other specific ages. Calves within an experimental grouping showed significant changes in the blood plasma vitamin A levels with age and among themselves (table 2).

The liveweight gains, as shown in table 3, were significantly higher for those calves from dams fed either form of vitamin A than for those calves from dams fed alfalfa leaf meal or dams fed the basal ration alone. In addition, calves from dams fed alfalfa leaf meal were significantly heavier in liveweight than calves from control dams. At any one age, the only statistical difference was at 4 weeks of age when calves from dams fed the ester form of vitamin A were significantly heavier in liveweight than calves from control dams (table 3).

TABLE 3
The effect of the perpartum diet on the liveweight of young calves

Breed	No. of calves	Age				
		Birth	1 week	2 weeks	3 weeks	4 weeks
		(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
<i>Controls</i>						
Holstein	14	95 ± 4	95 ± 4	98 ± 4	101 ± 4	108 ± 4
Brown Swiss	2	98 ± 2	98 ± 3	100 ± 1	106 ± 1	113 ± 0
Guernsey	2	68 ± 22	67 ± 23	66 ± 22	73 ± 28	78 ± 30
Jersey	1	56	56	59	63	72
All Breeds	19	90 ± 4	90 ± 4	92 ± 4	96 ± 4	103 ± 4
<i>Alfalfa leaf meal</i>						
Holstein	9	98 ± 3	99 ± 3	102 ± 3	108 ± 4	117 ± 4
Brown Swiss	2	80 ± 10	88 ± 16	83 ± 20	92 ± 23	100 ± 20
Guernsey	2	83 ± 6	85 ± 8	87 ± 9	86 ± 15	98 ± 9
Jersey	1	48	49	54	56	57
All Breeds	14	90 ± 4	92 ± 4	94 ± 5	99 ± 4	107 ± 6
<i>Alcohol form of vitamin A</i>						
Holstein	5	102 ± 6	107 ± 6	108 ± 6	115 ± 8	126 ± 10
Brown Swiss	1	77	79	85	103	104
Guernsey	2	74 ± 2	76 ± 3	80 ± 5	86 ± 3	91 ± 7
Jersey	1	55	55	47	56	65
All Breeds	9	88 ± 7	91 ± 7	92 ± 8	101 ± 8	109 ± 9
<i>Ester form of vitamin A</i>						
Holstein	11	98 ± 2	101 ± 3	107 ± 3	115 ± 3	124 ± 4
Brown Swiss	2	86 ± 1	87 ± 1	88 ± 1	93 ± 1	103 ± 1
Guernsey	1	80	81	89	95	99
Jersey	2	54 ± 10	59 ± 11	62 ± 12	69 ± 12	76 ± 14
All Breeds	16	90 ± 4	93 ± 4	98 ± 5	105 ± 5	114 ± 5

There were no significant differences found in the feed consumed by the various grouping of calves (table 4). The incidence of scours (days with scours) was significantly lower in those calves from dams fed either form of vitamin A than that observed in calves from basal dams. Calves from dams fed alfalfa leaf meal had significantly fewer days of scours than calves from basal dams (table 4).

DISCUSSION

These data furnish additional evidence of the importance of the prepartum ration on the subsequent performance of the newborn calf. Not only does the prepartum ration influence the storage of vitamin A in the newborn calf and in colostrum (21, 22, 25), but the combination of these two factors apparently

results in superior performance of the young calf as evidenced by the data presented in this paper. Similar data showing statistically higher blood plasma levels and liver storage levels have been found in 30-day-old pigs and lambs from dams fed supplementary vitamin A during the prepartum period (2).

The marked absence of response when carotene in the form of alfalfa meal is added to the maternal ration, in contrast to that found when either the ester or alcohol form of vitamin A is added, is noteworthy. Although earlier data (21, 22) have shown that the feeding of carotene *per se* does influence the carotene content of colostrum and the fetal storage of carotene, no appreciable in-

TABLE 4
Effect of the prepartum diet on the consumption of milk, starter and hay and the incidence of scours in young calves

Breed	No. of calves	Milk	Starter	Hay	Scours
		(lb.)	(lb.)	(lb.)	(days)
<i>Controls</i>					
Holstein	14	224.5 ± 5.8	12.5 ± 1.8	4.3 ± 0.9	4.4 ± 1.2
Brown Swiss	2	222.5 ± 6.5	16.0 ± 0.1	8.8 ± 5.7	3.5 ± 0.5
Guernsey	2	154.0 ± 25.0	10.4 ± 0.2	4.8 ± 4.7	7.5 ± 0.5
Jersey	1	165	15.0	5.0	2
All Breeds	19	213.7 ± 7.4	13.3 ± 1.3	4.9 ± 0.9	4.5 ± 0.9
<i>Alfalfa leaf meal</i>					
Holstein	9	238.2 ± 3.0	17.8 ± 2.9	4.8 ± 1.1	1.6 ± 0.7
Brown Swiss	2	218.5 ± 24.5	14.8 ± 0.3	7.3 ± 3.2	5.0 ± 5.0
Guernsey	2	158.5 ± 3.4	14.9 ± 8.1	2.7 ± 0.4	3.5 ± 3.5
Jersey	1	153.0	18.0	5.5	13.0
All Breeds	14	218.0 ± 9.6	17.0 ± 1.9	4.9 ± 0.8	3.1 ± 0.7
<i>Alcohol form of vitamin A</i>					
Holstein	5	223.4 ± 9.0	14.0 ± 2.4	8.0 ± 3.3	1.2 ± 1.0
Brown Swiss	1	244.0	9.5	7.5	0.0
Guernsey	2	149.5 ± 9.5	12.4 ± 4.8	5.5 ± 0.5	3.0 ± 3.0
Jersey	1	139.0	12.2	4.5	4.0
All Breeds	9	199.9 ± 14.6	12.9 ± 1.6	7.4 ± 1.5	2.2 ± 1.1
<i>Ester form of vitamin A</i>					
Holstein	11	242.5 ± 2.8	14.2 ± 2.3	4.8 ± 0.9	0.4 ± 0.4
Brown Swiss	2	236.5 ± 6.0	14.8 ± 4.2	9.9 ± 6.1	0.0 ± 0.0
Guernsey	1	161.0	16.1	11.0	0.0
Jersey	2	168.0 ± 0	17.2 ± 9.3	5.2 ± 0.7	0.0 ± 0.0
All Breeds	16	227.3 ± 7.9	15.4 ± 1.9	5.9 ± 1.0	0.3 ± 0.1

crease was noted in the vitamin A content as such. Later work by Fountain *et al.* (4) showed that calves from cows on a ration limited to pasture alone had blood plasma vitamin A values equivalent to those obtained in calves from dams fed grain, silage, hay and a vitamin A supplement. Recent studies (1, 7, 16, 19, 20, 24) have suggested that vitamin A is utilized more readily by dairy calves than is carotene. Also, the absorption and utilization of carotene is influenced by the source of carotene and the method of feeding. Crude soybean lecithin recently has been demonstrated (3) to influence the absorption and utilization of carotene and vitamin A in the young dairy calf. Clearly, this whole field needs further study.

The same response of calves to either the ester or alcohol forms of vitamin A fed to their dams is in agreement with other published information. Ross *et al.* (16) found no appreciable difference in blood plasma levels of vitamin A in Holstein heifers when the ester or alcohol form of vitamin A was fed in equivalent amounts. Regardless of the ration or the form of the supplementary vitamin A fed during the prepartum period, practically all of the vitamin A contained in the colostrum or milk was present in the ester form (14, 22). The prepartum feeding of supplementary vitamin A resulted in increases in the ester form of vitamin A occurring in the blood plasma (15). The increase in the ester form of vitamin A occurred when either the ester form or alcohol form of vitamin A was fed. In newborn and young calves the blood plasma vitamin A was predominately in the alcohol form, while the vitamin A contained in the liver was largely in the ester form. Limited data indicated that these relationships existed regardless of the form of supplementary vitamin A fed the dam.

The apparent absence of beneficial results from postnatal supplementary vitamin feeding (5, 6, 12, 13, 18) under so-called normal feeding conditions and the beneficial results reported herein in prepartum vitamin A supplementation would indicate the need for additional work with emphasis on prepartum nutrition. However, the limited number of calves, especially as to breeds other than Holsteins, would indicate the need for additional work before application of these results can be realized.

SUMMARY

The effects of feeding 1 million I.U. of vitamin A, in the form of dehydrated alfalfa leaf meal, the ester form of vitamin A and the alcohol form of vitamin A, daily to cows 30 days prepartum on the blood plasma levels of carotene and vitamin A, liveweight changes, feed consumption and incidence of scours of calves from birth to 28 days of age have been studied.

Carotene levels in the blood plasma of calves from dams fed the alcohol form of vitamin A were significantly higher during the experimental period than calves from dams in the other dietary groupings. Also, calves from dams receiving the ester form of vitamin A were significantly higher in plasma carotene than calves from dams fed alfalfa leaf meal.

Plasma of calves from cows fed vitamin A as either ester or alcohol was significantly higher in vitamin A than that of calves from the basal dams or those fed alfalfa leaf meal.

Greater liveweight increases were observed in calves from dams fed either form of vitamin A than in calves from dams fed the basal ration alone or with alfalfa leaf meal. In addition, calves from dams fed alfalfa leaf meal made greater increases in liveweight during the entire experimental period than calves from basal dams.

There were no statistical differences found in feed consumption.

Incidence of scours was significantly lower in calves from dams fed either form of vitamin A than that observed in calves from control dams. Calves from dams fed alfalfa leaf meal had fewer cases of scours than calves from control dams.

ACKNOWLEDGMENTS

The authors are grateful to Messrs. C. R. Richards and S. T. Slack for their aid in collecting much of the experimental data and determination of blood plasma carotene and vitamin A values and to Dr. R. W. Bratton for aid in the statistical analyses of the data. Further acknowledgement is made of the assistance of Mr. Paul Dean in the care of the experimental animals.

REFERENCES

- (1) BOYER, P. D., PHILLIPS, P. H., LUNDQUIST, N. S., JENSEN, C. W., AND RUPEL, I. W. Vitamin A and Carotene Requirements for the Maintenance of Adequate Blood Plasma Vitamin A in the Dairy Calf. *J. Dairy Sci.*, **25**: 433-440. 1942.
- (2) EATON, H. D., CHRISTIAN, J. A., DAUGHERTY, F. C., SPIELMAN, A. A., AND MATTERSON, L. D. The Effect of Crude Soybean Lecithin on the Absorption and Utilization of Vitamin A Fed Prepartum to the Ewe and Sow. *J. Animal Sci.*, in press.
- (3) ESH, G. C., SUTTON, T. S., HIBBS, J. W., AND KRAUSS, W. E. The Effect of Soya-Phosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, **31**: 461-478. 1948.
- (4) FOUNTAIN, F. C., PARRISH, D. B., ATKESON, F. W., AND HUGHES, J. S. The Effect of a Ration Limited to Pasture During the Dry-Rest Period on the Vitamin A and Carotenoid Content of the Blood of the Newborn Calf (Abs.). *J. Animal Sci.*, **7**: 525. 1948.
- (5) GILMORE, L. O., JONES, L. M., KANEGIS, L. A., AND ROEPKE, M. H. The Prophylactic Administration of Vitamins to Dairy Calves. *J. Am. Vet. Med. Assoc.*, **110**: 390-393. 1948.
- (6) HIBBS, J. W., AND KRAUSS, W. E. The Effect of Supplementary Vitamins on Blood Composition, Liver Storage, and Incidence of Scours in Calves. *J. Dairy Sci.*, **30**: 115-120. 1948.
- (7) JACOBSON, N. L., WISE, G. H., AND ALLEN, R. S. Effect of Administration of Carotene and of Vitamin A upon the Rate at which they are Absorbed From the Alimentary Tract of Dairy Calves. *J. Dairy Sci.*, **31**: 671. 1948.
- (8) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, **24**: 1055-1065. 1939.
- (9) LOVE, H. H. Experimental Methods in Agricultural Research. *Agr. Expt. Sta. Univ. Puerto Rico, Puerto Rico*. 1943.
- (10) MOORE, L. A., AND FLY, R. Extraction of Carotene from Plant Material. A Rapid Quantitative Method. *Ind. Eng. Chem. Anal. Ed.*, **13**: 600-601. 1941.
- (11) NELSON, W. L., LOOSLI, J. K., LOFGREEN, G., AND YAGER, N. Carotene Retention in Alfalfa Meal. Effect of Blanching, Packaging, and Storage Temperature. *Ind. Eng. Chem.*, **40**: 2196-2198. 1948.
- (12) NEVENS, W. B., AND KENDALL, K. A. The Value of Supplementary Vitamin Feeding in the Rearing of Dairy Calves. *J. Dairy Sci.*, **30**: 175-181. 1947.
- (13) NORTON, C. L., EATON, H. D., LOOSLI, J. K., AND SPIELMAN, A. A. Controlled Experiments on the Value of Supplementary Vitamins for Young Calves. *J. Dairy Sci.*, **29**: 231-238. 1946.
- (14) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S. The State of Vitamin A in Colostrum and in Milk. *J. Biol. Chem.*, **167**: 673-678. 1948.
- (15) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S. Effect of Vitamin A Supplements Upon the State of Vitamin A in Blood Serum of the Dairy Cow and in the Blood Serum and Liver of the Neonatal Calf. *J. Biol. Chem.*, **172**: 355-365. 1948.
- (16) ROSS, R. H., KNOTT, C. B., AND GUERRANT, N. B. The Utilization of Carotene, Vitamin A Alcohol and the Natural Ester of Vitamin A by Holstein Heifers. *J. Dairy Sci.*, **31**: 111-117. 1948.

- (17) SNEDECOR, G. W. Statistical Methods. 4th ed. Iowa State College Press, Ames. 1946.
- (18) SPIELMAN, A. A., DALEYMPLE, D. B. H., NORTON, C. L., LOOSLI, J. K., AND TURK, K. L. The Value of Supplementary Vitamins for Calves Raised Under Farm Conditions. *Am. J. Vet. Research*, 9: 26-29. 1948.
- (19) SPIELMAN, A. A., EATON, H. D., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. Unpublished data. Dept. Animal Husb., Cornell Univ. 1947.
- (20) SPIELMAN, A. A., LOOSLI, J. K., THOMAS, J. W., NORTON, C. L., AND TURK, K. L. Carotene Utilization in the Newborn Calf. *J. Dairy Sci.*, 29: 381-391. 1946.
- (21) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. The Placental Transmission and Fetal Storage of Vitamin A and Carotene in the Bovine. *J. Dairy Sci.*, 29: 707-715. 1946.
- (22) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., WHITING, F., NORTON, C. L., AND TURK, K. L. The Relationship of the Prepartum Diet to the Carotene and Vitamin A Content of Bovine Colostrum. *J. Dairy Sci.*, 30: 343-350. 1947.
- (23) TURK, K. L. Feeding Dairy Calves and Heifers. *Cornell Ext. Bull.* 361. 5th printing. 1941.
- (24) WARD, R. E., BECHDEL, S. I., AND GUERRANT, N. B. Carotene and Vitamin A in the Nutrition of Growing Dairy Cattle. *J. Dairy Sci.*, 23: 115-124. 1940.
- (25) WISE, G. H., CALDWELL, M. J., AND HUGHES, J. S. The Effect of the Prepartum Diet of the Cow on the Vitamin A Reserves of Her Newborn Offspring. *Science*, 103: 616-618. 1946.

THE RELATIONSHIP BETWEEN TYPE RATINGS OF AYRSHIRE FEMALES AS YOUNG HEIFERS AND AS COWS¹

GEORGE HYATT, JR.,² W. J. TYLER,³ AND C. T. CONKLIN⁴

The type and over-all eye appeal of an animal always has been an important factor in selecting breeding stock. Livestock breeders like to compare the type of their cattle with that of their neighbors at home, at fairs, and at shows and exhibits. Many breeders and educators argue that the type conformation of a dairy animal bears little relationship to her usefulness as a producer. They argue that a level rump, a clean-cut appearance and a well balanced and attached udder are of little importance in selecting and breeding cattle. Some have said that the show ring has done more harm than good in the development of our breeds. Others breeders and educators argue just as strongly that type and production go hand in hand, and stress proper type conformation, even to the point where some production may be sacrificed. Despite the arguments each way, the fact remains that dairymen and livestock breeders of all kinds usually try to seek the best type cows for additions to their herds and are willing to pay higher prices for these "typy" animals. Type classification programs have been adopted by the five major dairy cattle breed associations and, although these programs still need to be strengthened, they represent the most scientific approach yet devised for the estimation and use of type in a breeding program.

A study of the inheritance of official type ratings of Ayrshire cows was made by Tyler and Hyatt (3), who found heritability to be approximately 30 per cent. These same workers found the repeatability between type ratings given to the same cow by different inspectors to be 0.55. When the ratings on a cow were made by the same inspector the repeatability figures were 0.73, 0.82 and 0.62 for three inspectors.

Johnson and Lush (2) studied yearly type ratings of Holstein-Friesian females from 6 months of age to maturity. They state that "ratings made under one year of age were somewhat less repeatable than those made at older ages" and that "the increase in permanency of ratings after one year of age was small." When they omitted ratings on heifers less than one year of age, they reported a correlation of 0.34 between consecutive ratings when the ratings were made by nationally-known dairy cattle judges.

EXPERIMENTAL PROCEDURE

If type is heritable and important to breeders of dairy cattle, it is desirable that dairymen should be able to cull the poorest type animals from their herds

Received for publication January 6, 1949.

¹ Published with the approval of the Director, West Virginia Agricultural Experiment Station, as Scientific Paper no. 402.

² Professor of Dairy Husbandry, West Virginia University, Morgantown.

³ Assistant Professor, Department of Dairy Husbandry, West Virginia University, Morgantown.

⁴ Executive Secretary, Ayrshire Breeders' Association, Brandon, Vermont.

at as early a date as possible to save feed, labor and money. This raised the question as to whether the type classification program, as applied to cows, also could be used for evaluating the type of young heifers. Therefore, a cooperative project was inaugurated in 1942 by the West Virginia Agricultural Experiment Station and the Ayrshire Breeders' Association to determine the repeatability of heifer classifications at 6-month intervals and the correlation between the several classifications of a heifer and her classification after freshening. The Ayrshire cattle at the West Virginia Agricultural Experiment Station are known as the Reyman Memorial Herd and have been on a long-time, carefully controlled breeding project for more than 25 years. This project does not permit the culling of any normal females until they have completed at least one lactation.

During the period from October, 1942, through April, 1948, 102 Ayrshire heifers were classified every 6 months, starting at about 6 or 12 months of age and continuing in some instances to 5 years of age. The type ratings were made in each instance by an official inspector designated by the Ayrshire Breeders' Asso-

TABLE 1

The average number, mean and standard deviation of ratings on 102 Ayrshire females classified as heifers and as cows

	Ratings made as:		
	Heifers	Cows	Combined
Number of animals	102	102	102
Average number of ratings	4.0	2.4	6.4
Mean	3.15	3.09	3.13
Intra-animal standard deviation	0.62	0.65	0.66

ciation. The inspectors classified each heifer into one of five grades (Excellent, Very Good, Good Plus, Good and Fair) similar to the classification program of adult animals. In no instance did they have knowledge of the previous classification of the animals until the entire job had been completed. In order to facilitate the analysis of the data the official grades were coded, using 5 for Excellent, 4 for Very Good, 3 for Good Plus, 2 for Good, and 1 for Fair.

RESULTS

Variations in individual ratings of heifers and cows. The 102 females in the study had a total of 407 ratings before calving and 243 ratings following calving. These two groups of ratings were analyzed by the analysis of variance and the intra-animal standard deviations were computed. The results are shown in table 1. The variation in the ratings of animals before first calving was approximately the same as the variation in ratings given to the same animals after they had calved. These standard deviations also are similar to the intra-cow variation (standard deviation = 0.64) reported by Hyatt and Tyler (1) for 101 Ayrshire cows classified two times or more.

In terms of range (difference between highest and lowest ratings on an animal) 77 of the heifers, or 75 per cent, varied one grade or none from the time they

first were classified until first freshening, while 57 animals, or 60 per cent, varied one grade or none in their ratings from 6 months to 4 or 5 years of age. Before first calving 24 per cent of the animals had a range of two grades in their ratings, while 38 per cent of the animals showed a range of two grades when all ratings were considered. Only one heifer varied three grades before first calving, while six animals varied three grades for all ratings.

A review of the classification ratings of the six animals that ranged three grades showed that one heifer (no. 789) was classified Very Good at 12 months of age, Good Plus at 18 and 24 months, Fair at 30 months, and Good following freshening. Each time she was classified her feet and legs were Fair or Good and her final rating depended upon how severe a cut the inspector thought should be placed on that defect. Heifer no. 737 classified Good Plus at 6 and 18 months, Very Good at 12, 24 and 30 months. The first two times following freshening she classified Very Good, then dropped to Good and 6 months later classified Excellent. At the time she was classified Good she was dry and showed very little udder development. However, during heavy lactation following freshening, she showed a large and well-balanced udder and was classified Excellent. Heifer

TABLE 2

*Average classification score of 33 heifers at 6, 12, 18 and 24 months
and 73 heifers at 12, 18 and 24 months of age*

No. of heifers	Average classification rating			
	6 mo.	12 mo.	18 mo.	24 mo.
33	2.91	3.12	3.10	3.06
73		3.15	3.18	3.21

no. 729 classified Very Good at 12 months, Good Plus at 18 months and Good at 24 months. Following freshening, she was classified twice as Fair before leaving the herd. In each instance she was criticized for lacking in fore udder development and attachment. This fault appeared to become progressively worse as she became older. Heifer no. 727 classified Good Plus at 6 and 30 months, Very Good at 12 months and Good at 24 months. The first time after freshening she classified Good Plus but was dropped to Fair at the next classification. In each instance she was criticized for being sickle-hocked, and this condition became more defective as she became older. The other two animals that varied three grades were criticized for similar faults.

Effect of age on average type ratings of heifers. There were 33 heifers that were classified at 6, 12, 18 and 24 months of age. The averages of the ratings at the four different ages were approximately the same. This also was true of a group of 73 heifers classified at 12, 18 and 24 months of age. The results are shown in table 2.

These results seem to be contrary to the popular belief of many dairymen that most heifers appear to look typier at about 6 months of age than at any other time before freshening.

A comparison of the classification ratings of heifers before first calving with

TABLE 3

The correlation coefficients (r) between the various classification ratings of heifers at 6, 12, 18, 24 and 30 months of age and between these ratings and the 1st, 2nd and average of 1st and 2nd after-calving (AC) ratings

Age	Age					
	6 mo.	12 mo.	18 mo.	24 mo.	30 mo.	All before calving
	No. r	No. r	No. r	No. r	No. r	No. r
12 months	42 .30					
18 months	39 .10	89 .47 ²				
24 months	42 .33 ¹	92 .31 ²	94 .38 ²			
30 months	20 .40	66 .48 ²	67 .57 ²	73 .54 ²		
1st AC	42 .33 ¹	92 .31 ²	93 .22 ¹	102 .25 ¹	73 .35 ²	102 .37 ²
2nd AC	29 .23	67 .27 ¹	69 .29 ¹	76 .18	60 .45 ²	76 .40 ²
Av. 1st and 2nd AC	29 .35 ¹	67 .36 ²	69 .40 ²	76 .29 ²	60 .40 ²	76 .44 ²

¹ P < .05 > .01

² P < .01

their ratings after freshening. Correlation coefficients were computed between the various ratings before calving and also between the ratings before first calving and the ratings after calving. These coefficients are shown in table 3. Because of the limited number of animals in this experiment, the sampling errors are large. Thus, no particular age group had a significantly higher value for predicting after-calving ratings. The correlation between the average of the several ratings of each heifer before first calving and her first and second ratings after calving were 0.37 and 0.40, respectively. This is slightly smaller than the repeatability figure of 0.55 between the ratings of cows classified by different inspectors (1).

The regression of average after-calving ratings on the average ratings before calving was 0.45. An example of the use of this regression may be shown as follows: The type ratings of the heifers before first calving were compared with their ratings after calving by placing all the heifers in groups according to their average classification score prior to first calving. This average group score before freshening¹ was then compared with the average classification score of the same group after first calving. The average score was computed by giving each animal 92.5 points for each Excellent rating, 87.5 for Very Good, 82.5 for Good

TABLE 4

Average classification ratings by groups before and after calving

Average rating of animal before calving by groups	No. of animals	Average of each group before calving	Average of each group after calving
Below 80.5	14	78.6	80.1
80.5-82.5	34	82.0	81.5
82.6-85.0	31	84.4	83.0
Above 85.1	23	87.1	84.5
Total	102	83.4	82.4

Regression of average after-calving rating on average before-calving rating = 0.45.

Plus, 77.5 for Good, and 72.5 for Fair, and then adding up the points and dividing by the total times classified. Those animals that averaged below 80.5 were placed in one group, those whose average ratings were between 80.5 and 82.5 fell in the second group, while the other two groups were comprised of animals whose average classification scores were between 82.6 and 85.1 and higher. The average of each group before calving is given in table 4 and can be compared with the group average of the same animals after they have freshened. The heifers that were in the two lower groups, as far as type was concerned, still had the lower classification average after they were rated as milking cows, but the averages were closer to the average of all the animals. Those animals in the upper groups were still above average when they were classified as 3-, 4- and 5-year old cows, but the two group averages were nearer the average of all groups.

DISCUSSION

The results of this study have indicated that the relationship between any classification rating on Ayrshire heifers before calving and a rating after calving is about 0.3. In addition, correlations between combinations of ratings before first calving and ratings after calving are approximately 0.4. In a previous study on this herd (1) the repeatability of classification ratings for the same cow was estimated to be 0.55. Thus, while ratings on heifers before first calving are indicative of after-calving ratings, they are not as reliable as after-calving ratings in predicting other after-calving ratings. These results are similar to the study on classifications of Holsteins reported by Johnson and Lush (2).

The variation between ratings on the same animal before calving was similar to the variation that was found between ratings on the same cow. This presents the same problem in classifying heifers as was found in the classification of cows (1), namely, a means of identifying and adjusting for those factors which cause ratings to vary during the life of the animal. Since nine different inspectors classified the animals during the course of this experiment, an undetermined part of the variation in the ratings was caused by differences of opinion of the inspectors. In other instances, the differences in ratings were caused by changes in the condition and/or type of the animals. At the present time the basis used for the classification of uncalved heifers is the same that has been applied in classifying cows. This may be the wrong approach in classifying heifers. Research on the development of the features of type in growing heifers may reveal the essential parts of the heifer that contribute to her type as a mature animal. Perhaps with more experimental work, detailed records and frequent consultations on the part of inspectors with one another, it may be possible to reduce the variation in heifer classification that is due to differences in inspectors and to inaccuracies in weighing the good and poor points of heifers during their early development.

The standard deviation of all ratings on the heifers before their first calving was 0.78. This is smaller than the standard deviation (0.94) for all ratings on cows in the same herd during the same period. Part of the smaller variation

in ratings on heifers may be attributed to the observation that heifers generally do not show the extreme faults in feet, legs and udders that cows do. In addition, many inspectors hesitate to classify heifers Fair or to classify them Excellent because later development of the heifer may make their decision look absurd.

SUMMARY

One hundred and two Ayrshire heifers at the West Virginia Agricultural Experiment Station have been rated for type starting at 6 or 12 months of age and at 6-month intervals thereafter until 4 or 5 years of age. The intra-animal standard deviation was 0.62 when the ratings were made before first calving, and 0.65 when they were given after calving.

In terms of range, five animals (4.9 per cent) were classified the same whether rated as heifers or milking cows, 52 (51.0 per cent) varied one grade, while 39 (38.2 per cent) varied two grades and six (5.9 per cent) varied three grades.

There were 33 heifers classified at 6, 12, 18 and 24 months of age and 73 heifers classified at 12, 18 and 24 months. The average of the ratings at the different ages was approximately the same.

The correlations between the average of the several ratings of each heifer before first calving and the first and second ratings after calving were 0.37 and 0.40, respectively.

The average type ratings of the heifers before calving were compared with the average ratings after calving by placing the heifers in four groups (below 80.5, 80.5 to 82.5, 82.6 to 85.0, above 85.1) according to the average of their ratings before first calving. The low group remained the lowest following calving, while the high group still maintained the highest average score, but both averages were closer to the general mean. The two middle group averages changed very little following freshening.

With improvement in and standardization of classification methods, particularly for heifers, the classification program may become valuable in helping breeders to cull the poorest type individuals from their herds at an early age.

REFERENCES

- (1) HYATT, GEORGE, JR., AND TYLER, W. J. Variations in Type Ratings of Individual Ayrshire Cows. *J. Dairy Sci.*, 31: 71-79. 1948.
- (2) JOHNSON, LESLIE E., AND LUSH, JAY L. Repeatability of Type Ratings in Dairy Cattle. *J. Dairy Sci.*, 25: 45-56. 1942.
- (3) TYLER, W. J., AND HYATT, GEORGE, JR. The Heritability of Official Type Ratings and the Correlation Between Type Ratings and Butterfat Production of Ayrshire Cows. *J. Dairy Sci.*, 31: 63-70. 1948.

ASSOCIATION ANNOUNCEMENT

FORTY-FOURTH ANNUAL MEETING
UNIVERSITY OF MINNESOTA
JUNE 21-23, 1949

REGISTRATION AND HOUSING

Registration headquarters will be in Coffman Memorial Union, Minneapolis Campus. Rooms to accommodate about 250 persons will be available in dormitories on the St. Paul Campus. Families with children will have prior claim to these rooms. Approximately 400 rooms have been reserved in Twin City hotels. A return card relative to advance registration and housing will be sent to members by the Association Secretary in May.

LOCATION OF GENERAL AND SECTION MEETINGS

All meetings will be held in or in close proximity to Coffman Memorial Union, Minneapolis Campus, University of Minnesota.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings and committee meetings before, during or after the regular sessions will please contact Elmer L. Thomas of the Division of Dairy Husbandry, University Farm, St. Paul. Reservations for special breakfasts and luncheons will be made upon request. It will be appreciated if all such requests are made known by June 1.

PROJECTION EQUIPMENT

While it is desired that the use of slides be minimized, projectors for both standard and 2" x 2" slides will be available in all lecture rooms. Requests for use of such equipment should be made to section chairmen as soon as possible.

ADDITIONAL INFORMATION

Additional material containing information on registration, housing, entertainment, recreation, etc., will be sent to each member about April 15, 1949.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

MAY, 1949

NUMBER 5

STALE FLAVOR COMPONENTS IN DRIED WHOLE MILK. I. THE DISTRIBUTION OF STALE FLAVOR BETWEEN FRACTIONS OF RECONSTITUTED STALE WHOLE MILK POWDER

R. McIL. WHITNEY AND P. H. TRACY

Department of Food Technology, University of Illinois, Urbana, Illinois

During storage a stale flavor often develops in dried whole milk which is very objectionable and reduces the consumer's acceptance of the product. Considerable research has been done to prevent or retard the development of this stale flavor by means of variations in the method of manufacture (6) and also to correlate the occurrence of this off-flavor with certain chemical changes observed in the powder (1).

However, neither the exact chemical compound responsible for the stale flavor nor its mechanism of formation are known. As a step toward the isolation and identification of the stale-flavor component, its distribution between fractions of reconstituted stale whole milk powder was investigated.

EXPERIMENTAL

Manufacture and storage of dried whole milk. Whole milk was condensed, dried in a pilot-size experimental spray drier and stored under varying conditions in order to have a continuous supply of stale dried whole milk for this study. All lots of powder were manufactured from milk without previous homogenization and at low spray pressures (450–700 p.s.i.) to facilitate the subsequent fractionation of the reconstituted stale whole milk.

Method for determining the relative concentrations of the stale-flavor component in the fractions. The relative concentration of the stale-flavor component in each of the fractions was determined by establishing *the threshold concentration of the stale fraction* when blended with an appropriate non-stale product to the approximate composition of the original whole milk. Since this judging technic has not been reported before for flavors in milk, it is described in detail as follows with stale cream as an example:

1. A reconstituted milk of approximately the same composition as the original milk with respect to fat and total solids was prepared from the stale cream and non-stale skim milk in a malted-milk mixer at 43° C. by agitating for 1 minute.

2. Two series of five samples each, of overlapping concentrations with respect to the percentage of stale cream in each of the samples, were prepared by diluting this reconstituted milk with non-stale whole milk of the same composi-

Received for publication September 7, 1948.

tion. For example, the five samples in the first series might contain 3, 5, 7, 10 and 16 per cent stale cream, while the second series might contain 1, 4, 6, 8 and 14 per cent stale cream.

3. The samples were tempered at 24° C. in a water bath (approximately 10 minutes).

4. A panel of experienced judges tasted the samples, arranged at random in each series, and recorded those in which they could detect the stale flavor.

5. The judgments of each judge on each series and on the combined series were arranged in the order of the concentrations of stale cream, and the lowest consistent concentration in each series at which the stale flavor was detected was selected as the threshold value. If a judge recorded one sample inconsistently, the lowest consistent positive judgment was selected as his threshold value for the series. If he recorded two samples inconsistently, his judgment was rejected on that series. Table 1 illustrates this procedure.

TABLE 1
A sample determination of the threshold values of three judges

Sample no.	Stale cream (%)	Judgments		
		1st judge	2nd judge	3rd judge
1-2	16	+	+	-
1-4	10	+	+	+
1-5	7	+	-	-
1-3	5	-	-	+
1-1	3	-	+	-
Threshold value of stale cream (%)		7	10	rejected

6. From all the judgments of all the judges, the mean threshold value and the standard deviations were calculated. This mean threshold value bears a reciprocal relationship to the concentration of the stale-flavor component in the stale cream.

The effect of separation of whole milk upon the distribution of stale-flavor component. Upon the development of sufficient stale flavor in the dried whole milk, the powder was reconstituted with distilled water to the composition of the original whole milk in a 10-gallon pilot-size pasteurizer constructed with a conical base. Agitation for 1 to 4 minutes at 43° C. with a motor-driven stirrer at 1,550 r.p.m. effected complete reconstitution. The milk then was separated into cream and skim milk with a motor-driven De Laval separator, model no. 18, at temperatures ranging from 12 to 64° C. and at rates of flow ranging from 2.3 to 6.2 quarts per minute, in order to determine both the optimum conditions for efficient separation of this product and the effect of these variables upon the distribution of the stale-flavor component between the cream and skim milk. The fractions then were cooled in an ice bath to approximately 10° C. and stored at this temperature until scored. Analyses of the fat content of the reconstituted whole milk, cream and skim milk were performed in duplicate by

TABLE 2
The effect of temperature and rate of flow during separation of reconstituted whole milk upon the distribution of staler-fat or component

Expt. no.	Sepa- ration temp.	Rate of flow	Fat tests			Threshold values for stale products ^a			Fat from stale products at threshold value		
			Milk	Cream	Skim milk	Milk	Cream	Skim milk	Milk	Cream	Skim milk
(°C.)	(qt./ min.)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	64	5.7	4.1	40.8	1.6	{ (2) not stale (6) 60 ± 10 }	{ (4) not stale (2) 6.0 ± 0.0 }	{ (2) not stale (2) 90 ± 0 }	{ not stale (2) 2.5 ± 0.4 }	{ not stale (2) 2.4 ± 0.0 }	{ not stale (2) 1.4 ± 0.0 }
2	63	5.8	4.2	42.0	1.2	(5) 60 ± 19	(8) 4.4 ± 1.5	(8) 42 ± 10	2.6 ± 0.8	1.8 ± 0.6	0.50 ± 0.12
3	43	6.2	4.3	40.0	2.35	(8) 67 ± 11	(5) 8.0 ± 1.0	(4) 60 ± 0	2.8 ± 0.5	3.2 ± 0.4	1.4 ± 0.0
4	43	5.9	4.1	38.0	2.3	(8) 52 ± 19	(8) 6.5 ± 1.3	(8) 48 ± 19	2.1 ± 0.8	2.5 ± 0.5	1.1 ± 0.5
5	43	5.9	4.0	40.0	1.8	(8) 52 ± 19	(6) 5.0 ± 0.4	(6) 47 ± 21	2.2 ± 0.8	2.0 ± 0.2	0.85 ± 0.32
6	43	5.7	4.2	37.5	1.6	(6) 50 ± 18	(8) 5.2 ± 1.0	(8) 38 ± 20	2.2 ± 0.8	2.0 ± 0.4	0.61 ± 0.32
7	43	5.4	4.3	40.5	2.0	(8) 55 ± 9	(4) 5.4 ± 0.3	(2) not stale	2.2 ± 0.1	2.2 ± 0.1	not stale
8	43	2.3	4.4	31.5 ^b	2.65	(6) 37 ± 3	(6) 8.3 ± 1.4	(8) 70 ± 17	2.4 ± 0.4	2.6 ± 0.4	1.9 ± 0.5
9	32	5.7	4.3	38.5	1.9	(6) 37 ± 3	{ (2) not stale (4) 3.4 ± 2.2 }	(6) 35 ± 16	1.6 ± 0.6	1.3 ± 0.8	0.66 ± 0.33
10	32	5.6	4.2	38.5	2.0	(10) 36 ± 9	(6) 4.0 ± 0.0	(8) 48 ± 15	1.5 ± 0.4	1.5 ± 0.4	0.93 ± 0.30
11	21 ^c	6.3	4.2	40.0	2.45		(5) 4.0 ± 0.9	(5) 27 ± 3	1.5 ± 0.4	1.6 ± 0.4	0.66 ± 0.07
12	12	5.3	4.2	38.5	2.7		(8) 4.8 ± 2.0	(8) 30 ± 13	1.8 ± 0.8	1.8 ± 0.8	0.81 ± 0.35

^a The numbers in parentheses indicate the number of judgments. Rejected judgments are not included.

^b No cream was obtained from cream spout during separation. This cream was obtained from the bowl after separator had stopped.

^c The reconstituted milk was held for 2 hr. at 4° C. and then warmed at 21° C. before separation.

means of the standard Babcock test, except in the case of the skim milk, for which the normal butyl alcohol modification of Hansen *et al.* (2) was used.

The reconstituted whole milk, cream and skim milk each were blended with appropriate non-stale products in the manner previously described. The results of the fat analyses and the judgments are given in table 2. Variations of the temperature of separation and the rate of flow apparently had little effect upon the distribution of the stale-flavor component between the cream and the skim milk. However, a more satisfactory separation was obtained at 43° C. with a normal rate of flow (6.0 quarts per minute) than under the other conditions investigated. Therefore, these conditions were maintained in subsequent work. Apparently, even though homogenization was avoided as much as possible during the manufacturing process, the reconstituted milk could not be separated with an efficiency at all comparable with that of commercial practice.

In all cases observed, the threshold value for the stale cream was lower than that of either the original stale reconstituted whole milk or the stale skim milk. Therefore, the stale-flavor component appeared to be more concentrated in the cream than in either the whole milk or skim milk.

TABLE 3
The effect of churning upon the distribution of the stale-flavor component

Product	Fat	Threshold value of stale product ^a	Fat from stale product at threshold value
	(%)	(%)	(%)
Cream	26.0	(2) 14.0 ± 0.0	3.6 ± 0.0
Butter	70.54	(6) 3.8 ± 0.5	2.7 ± 0.4
Buttermilk	3.1	(6) 43 ± 27	1.3 ± 0.8

^a The numbers within the parentheses indicate the number of judgments. Rejected judgments are not included.

The effect of churning of stale cream upon the distribution of stale-flavor component. The excess stale cream and skim milk obtained in a previous separation experiment were frozen at -26° C. on the same day that they were prepared. On the afternoon preceding the day of churning, they were removed and stored at 7° C. until the following morning. The melting then was completed in a 21° C. water bath, with the temperature of the product not rising above 10° C. The melted cream and skim milk then were blended to yield a product containing approximately 25 per cent fat and churned at 9 to 14° C. in a 1-gallon glass churn. The buttermilk was drained off and the butter chilled and washed at 7° C.

Fat analyses were performed in duplicate. Heinemann's modification of the Mojonnier method (3) was used in testing the butter, while the buttermilk was analyzed by the normal butyl alcohol modification of the Babcock test (2).

The cream, butter and buttermilk each were blended with the appropriate non-stale product and judged in the manner previously described. The results of the fat analyses and the judgments are given in table 3. A comparison of the threshold values of the stale fractions indicated that the stale-flavor com-

ponent was more concentrated in the butter than in either the cream from which it was churned or the buttermilk resulting from the churning.

The effect of washing of stale cream upon the distribution of stale-flavor component. Stale cream was prepared and mixed with sufficient distilled water to make the total weight equal to the weight of the reconstituted whole milk from which the cream was separated. After agitation in the pilot-sized pasteurizer with a motor-driven stirrer for 30 minutes at 1550 r.p.m. at 41 to 46° C., this mixture was separated immediately in the De Laval separator. This process was repeated four times. At the end of the fourth treatment, the cream had "oiled off" almost completely. Fat analyses were performed in duplicate by means of the standard Babcock test, except in the case of the wash water for which the normal butyl alcohol modification (2) was used.

The cream, washed cream and washed water each were blended with appropriate non-stale products and judged in the manner previously described. The experimental results are recorded in table 4. The washed stale cream had a

TABLE 4
Effect of washing stale cream upon the distribution of stale-flavor component

Product	Fat	Threshold value of stale product ^a	Fat from stale product at threshold value
	(%)	(%)	(%)
Cream	40.0	(6) 5.0 ± 0.4	2.0 ± 0.2
Washed cream	77.6	(10) 2.0 ± 0.7	1.6 ± 0.5
Wash water	0.07	(6) not stale at 60%	

^a The numbers within the parentheses indicate the number of judgments. Rejected judgments are not included.

lower threshold value than that of the original stale cream, while the wash water was reported as "not stale" when blended with non-stale condensed milk in the highest percentage possible without deviating from the composition of the original milk. Therefore the stale-flavor component appeared to be more concentrated in the washed cream than in either the original cream or the wash water.

The effect of preparation of stale butter oil upon the distribution of the stale-flavor component. Stale butter prepared in previous experiments was stored at -26° C. until used in this study. After the stale butter was melted at approximately 36 to 40° C. in a water bath, it was placed in solubility tubes and centrifuged in a heated Babcock centrifuge at 36 to 40° C. until a sharp boundary was obtained between the butter oil and butter plasma (10 to 30 minutes). The butter oil layer was decanted and filtered at 36 to 40° C. in an incubator. The butter plasma was analyzed for fat content in duplicate by the normal butyl alcohol modification of the Babcock test (2). The butter oil was assumed to be 100 per cent fat, since the available analytical methods would not yield reliable results within this range of fat concentration (4).

The butter, butter oil and butter plasma each were blended with appropriate non-stale products and scored in the manner previously described. These re-

sults are recorded in table 5. Some difficulties were experienced with the butter plasma fraction in these experiments. In the first experiment, the plasma apparently fermented during fractionation, while the amount of plasma obtained in the second experiment was so small that it was impossible to make up samples sufficiently concentrated to determine its threshold value.

However, the experimental results reported in table 5 indicate that the stale-flavor component is more concentrated in the butter oil than in either the original butter or the butter plasma when prepared under the conditions of this experiment. While a threshold value for the stale butter serum was not obtained, it was reported as "not stale" at concentrations higher than the threshold values for either the stale butter oil or stale butter.

TABLE 5

The effect of the preparation of butter oil upon the distribution of stale-flavor component

Product	Fat	Threshold value of stale products ^a	Fat from stale product at threshold value
	(%)	(%)	(%)
Expt. 1.			
Butter	70.54	(4) 3.5 ± 0.6	2.5 ± 0.4
Butter oil	100.0	(8) 3.0 ± 1.1	3.0 ± 1.1
Butter plasma	10.4	(8) Sour	
Expt. 2.			
Butter	83.35	(4) 4.0 ± 0.6	3.4 ± 0.5
Butter oil	100.0	(6) 2.8 ± 0.1	2.8 ± 0.1
Butter plasma	6.5	(3) Not stale at 6%	

^a The numbers within the parentheses indicate the number of judgments. Rejected judgments are not included.

DISCUSSION

As an aid in the further interpretation of the result of this study, the amount of stale fat from a stale product present in the sample at the threshold value, expressed as the percentage of the total weight of the sample, has been calculated for all of the stale fractions and recorded in their respective tables. For the reconstituted whole milk, cream, washed cream, butter and butter oil, these values are the same within each experiment, considering the limits of accuracy of the judgments. Therefore it appears that the stale-flavor component is distributed between these fractions according to their milk-fat content and is concentrated in the milk-fat phase.

In the skim milk and buttermilk, the stale-flavor component has a higher concentration per unit weight of fat than might be predicted from the above conclusion. Two possible explanations can be advanced for this observation. First, if the stale-flavor component were adsorbed on the surface of the fat globule, the amount adsorbed per unit weight of fat would be greater the larger the surface area of the fat or the smaller the fat globule. Since the fat globules in the skim milk and the buttermilk may be expected to be smaller than in the other fractions, the concentration of stale-flavor component per unit weight of fat should be higher in these fractions if this hypothesis is correct.

A second explanation for these results would be the assumption of an equilibrium distribution of the stale-flavor component between the fat and plasma phases. The larger percentages of plasma in the skim milk and buttermilk fractions, even though its concentration of stale-flavor component was very low, might cause a higher apparent value per unit weight of fat. However, until a more accurate method for measuring the concentration of stale-flavor component is available, a choice between the two hypotheses is difficult.

It is impossible to test the conclusion that the stale-flavor component distributes itself between the fractions in proportion to the fat content in the case of either the wash water or the butter plasma fractions, since the concentrations of stale fractions attainable were insufficient to produce a detectable stale flavor.

It should be pointed out that the evidence secured so far in this study does not demonstrate the fraction in which the stale-flavor component originates, but only the one in which it is concentrated upon fractionation by the procedure used. Therefore, it should not be considered as supporting or conflicting with any of the current opinions of its origin (1, 5).

One observation made during this investigation should be emphasized. In order to estimate the relative concentration of stale-flavor component in the various fractions, it is necessary to judge all fractions in blends of approximately the same composition, particularly with respect to their fat content. Evidently the higher the fat content of the sample tasted the less *intense* is the *stale-flavor sensation for the same concentration of stale-flavor component*. For example, when the cream and skim milk were tasted without blending, *the intensity of the stale-flavor sensation* was greater in the skim milk than in the cream but, upon dilution with non-stale product to the same composition, much less stale cream than stale skim milk was required in order to yield a detectable stale flavor, which indicates that *the stale-flavor component was more concentrated* in the cream than in the skim milk. This same phenomenon was observed in the cream-washing experiment in which the *intensity of the stale-flavor sensation* was greater in the wash water than in the washed cream. However, upon dilution with appropriate non-stale products to the same composition, a concentration of only 2 per cent washed cream was necessary to yield a detectable stale flavor, while, even at a concentration of 60 per cent wash water, no stale flavor could be detected, indicating that the *stale-flavor component was more concentrated* in the washed cream than in the wash water.

SUMMARY

Dried whole milk was prepared without homogenization and at low spray pressure in order to facilitate fractionation of the reconstituted milk. The powder was stored under varied conditions to insure a continuous supply of stale dried whole milk for this study.

Reconstituted stale dried whole milk was separated mechanically into cream and skim milk at various temperatures of separation and rates of flow. The cream was churned into butter and buttermilk or washed with distilled water until it oiled off. The butter was melted, centrifuged and filtered, all at 40° C., to yield butter oil and butter plasma.

The relative concentration of the stale-flavor component in the various fractions was determined by establishing the *threshold concentration of the stale fraction* when blended with an appropriate non-stale product to the approximate composition of the original whole milk.

In all determinations made on the whole milk, cream, washed cream, butter and butter oil, the stale-flavor component appears to be distributed between these fractions according to their milk-fat content and therefore is concentrated in the milk-fat phase.

ACKNOWLEDGMENT

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned no. 217 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Dept. of the Army.

The authors wish to express their appreciation to W. A. Krienke, R. J. McCauley, and O. M. Schreiter for aid in performing the experiments.

REFERENCES

- (1) COULTER, S. T., AND JENNESS, R. Some Changes in Dry Whole Milk During Storage. Ann. Rept., Q.M.C. Food and Container Research Inst. for the Armed Forces. File D-305, report no. 2.
- (2) HANSEN, H. C., HORRALL, B. E., BIRD, E. W., AND JENSEN, C. Final Report of the Subcommittee on the Determination of the Percentage of Fat in Buttermilk, Skim Milk and Whey. J. Dairy Sci., 28, 4: 325-327. 1945.
- (3) HEINEMANN, B. Methods of Testing 80 Per Cent Cream for Butterfat. J. Dairy Sci., 29, 10: 689-694. 1946.
- (4) HERREID, E. O., JENNESS, R., AND WHITMAN, D. W. A Study of the Ether Extract of the Materials Estimated as Fat in Babcock Test of Milk. J. Dairy Sci., 26, 9: 883-891. 1943.
- (5) HETRICK, J. H., AND TRACY, P. H. Some Observations on the Keeping Quality of Spray-Dried Whole Milk Stored at Room Temperature. J. Dairy Sci., 28, 9: 687-700. 1945.
- (6) TRACY, P. H., AND WILSTER, G. H. Prevention of the Development of Stale Flavor in Dry Whole Milk During Storage. Termination Rept., Q.M.C. Food and Container Research Inst. for the Armed Forces. File BD-3, report no. 4.

A SOLUBILITY METHOD FOR THE DETERMINATION OF ALPHA AND BETA LACTOSE IN DRY PRODUCTS OF MILK

R. P. CHOI, C. W. TATTER, C. M. O'MALLEY AND B. W. FAIRBANKS
The American Dry Milk Institute, Inc., Chicago, Illinois

Since the form of lactose in dry products of milk is important in determining the physical properties of the products, a procedure for estimating the two forms of lactose is desirable from the standpoint of controlling product uniformity. A polarimetric method was reported by Sharp and Doob (7). The present proposed method is based upon the difference in solubility behavior of *alpha* lactose hydrate and the *beta* anhydride, the two stable forms of lactose.

From the work of Hudson (4), an equilibrium is believed to exist between the *alpha* and *beta* forms of lactose in solution. The rate of attainment of equilibrium is slow and can be followed by solubility measurements. Hudson (4) found that when *alpha* lactose hydrate is added in excess to water, a definite amount will dissolve initially, and then more will go into solution slowly until a final solubility is attained. The rate of dissolution is independent of the concentration of the solid phase as long as an excess is present. The initial solubility is considered to be the equilibrium concentration of the *alpha* form, while the slower dissolution is interpreted to arise from the mutarotation of the *alpha* to the *beta* form so that the difference between the final and the initial solubility represents the equilibrium concentration of the *beta* form. On this basis both equilibrium and rate equations have been derived by Hudson (4).

Likewise, *beta* lactose exhibits an initial and final solubility. At 0° C. where data are available (5), the initial solubility of *beta* lactose is approximately eight times that of the *alpha* hydrate. The solubility of *alpha* lactose hydrate in milk was investigated by Hunziker and Nissen (6), who came to the conclusion that the constituents of milk have no effect on the solubility of *alpha* lactose hydrate. This, together with the wide difference in the initial solubility of the two forms of lactose, provides the basis for the present method. Because of the high initial solubility of *beta* lactose, it may be expected that in any mixture containing an excess of *alpha* lactose hydrate and a quantity of *beta* lactose less than its initial solubility, the total initial solubility will consist of all the *beta* plus the initial solubility of the *alpha* hydrate. From experiments with artificial mixtures containing different proportions of *beta* lactose, it has been found that this simple relationship is realized below a concentration of *beta* lactose of approximately 10 g. per 100 ml. of water, corresponding to a total initial solubility of 55 m.mols. per 100 ml. of water. Therefore, for any mixture such as a dry product of milk, the essential steps in the procedure would consist of: (a) adding *alpha* lactose hydrate in excess to a known weight of the mixture, (b) determining the solubility at several time intervals and (c) extrapolating the results to zero time to obtain the total initial solubility from which the amount of *beta* lactose in the mixture may be calculated easily. The

Received for publication December 9, 1948.

quantity of *alpha* lactose in the original mixture is the difference between the total lactose and the *beta* lactose.

In any extrapolation it always is desirable that the extrapolation be linear. In the present case this could be achieved by means of the equations derived by Hudson (4). When the concentration of lactose in solution is less than the final solubility, the solubility increases with time. The maximum rate of solution is given by equation I:

$$kt = \log \frac{S_{\infty} - S_0}{S_{\infty} - S_t},$$

where k is the rate constant; S_{∞} is the final solubility, which at 25° C. is 63.3 m.mols. per 100 g. of water; S_0 is the initial solubility of *alpha* lactose, or if *beta* lactose is present it is the total initial solubility; and S_t is the solubility at time t .

In a solution in which the concentrations of both the *alpha* and *beta* lactose are in excess of their equilibrium concentrations and in which *alpha* lactose hydrate also is present as a solid phase, Hudson (4) found that there is an immediate precipitation or crystallization of the excess *alpha* followed by a slow conversion of the excess *beta* to the *alpha* modification, which then crystallizes out as fast as it is formed. The maximum rate of crystallization under these conditions is given by equation II:

$$kt = \log \frac{C_0 - S_{\infty}}{C_t - S_{\infty}},$$

where k is the rate constant; S_{∞} is the final solubility; C_0 is the total initial solubility of the *alpha-beta* mixture; and C_t is the solubility at time t . Both equations I and II describe first order reactions. Therefore, if we plot in each case the log of the denominator, that is, $\log(S_{\infty} - S_t)$ or $\log(C_t - S_{\infty})$ against t , we should obtain a straight line whose slope is the velocity constant k and whose intercept is $\log(S_{\infty} - S_0)$ or $\log(C_0 - S_{\infty})$. The total initial solubility, which is S_0 or C_0 , can be calculated since the final solubility is known accurately from the work of Hudson (4).

EXPERIMENTAL PROCEDURE

Apparatus. The apparatus consisted of a Pyrex glass cylinder, 5.5 cm. in diameter and 15 cm. long, fitted with a two-hole rubber stopper. Through one hole an electrically-driven stirrer was inserted. The other hole was used as a sampling outlet and stoppered when not in use. The set-up was placed in a constant temperature water bath maintained at 25° C.

Total initial solubility with varying amounts of beta lactose. The proposed procedure first was tested using solid mixtures containing varying amounts of *beta* lactose and 30 to 40 g. of *alpha* lactose hydrate. With stirring applied, 100 ml. of distilled water at 25° C. was pipetted into the solubility chamber containing the solid mixture. At 10 minute intervals 10 to 15 ml. portions of the suspension were withdrawn and filtered immediately through a fritted glass filter of medium porosity with the aid of suction. An aliquot of the clear fil-

trate was analyzed for lactose by the Hinton-Macara method (3) and the solubility calculated as follows:

$$\text{m.mols. lactose/100 ml. H}_2\text{O} = \frac{\text{ml. Na}_2\text{S}_2\text{O}_3 \times \text{normality} \times 100}{2 \times \text{ml. filtrate} \times \text{water factor.}}$$

The water factor represents the fraction of water in the filtrate and can be determined by the toluol distillation procedure (1). The total initial solubility was calculated as described in the above section. The *beta* lactose content of the mixture then was plotted against the corresponding total initial solubility.

Procedure for dry products of milk. Except for roller process nonfat dry milk solids, 30 g. of the dry milk product generally were used. For roller process nonfat dry milk solids, only 25 g. could be used because of the high viscosity of the suspension. The sample was transferred to the solubility cylinder and mixed thoroughly with 30 g. of *alpha* lactose hydrate powder. To the solid mixture 100 ml. of distilled water at 25° C. were added and the time noted. Before transferring the flask to the water bath, it was necessary to free any material adhering to the sides and bottom of flask to insure rapid and complete dispersion. Stirring then was applied at a rate sufficient to keep the solids in suspension. At 10-minute intervals, 20–25 ml. of the suspension were withdrawn and immediately centrifuged for 3 minutes at approximately 1000 r.p.m. The centrifugate was decanted carefully and a 10-ml. portion used for lactose determination by the Hinton-Macara method (3). Total lactose also was determined for each dry milk sample. The solubility in m.mols. of lactose per 100 ml. of water was calculated as in the preceding section. When the above sample sizes were used, the total initial solubilities generally were less than 55 m.mols. per 100 ml. of water. Accordingly, $\log (63.3 - S_t)$ was plotted against the time and the best straight line drawn through the points. From the intercept at $t = 0$, the total initial solubility was calculated. The *beta* lactose in the quantity of sample taken for analysis was evaluated by subtracting from the total initial solubility the initial solubility of the *alpha* lactose which is 25.3 m.mols. per 100 ml. of water. Knowing the total lactose, the *alpha* modification was calculated by difference. Results are expressed as per cent of the total lactose.

RESULTS AND DISCUSSION

Figure 1 shows a plot of the total initial solubility against the quantity of *beta* lactose in a mixture containing an excess of *alpha* lactose hydrate. The curve ($\alpha + \beta$) is the experimentally determined curve; the curve β is calculated by assuming instantaneous dissolution of all *beta* lactose added. It can be seen from the parallelism of the two curves that below a concentration of 10 g. of *beta* lactose per 100 ml. of water, corresponding to a total initial solubility of about 55 m.mols. per 100 ml. of water, the total initial solubility is the sum of the initial solubility of the *alpha* hydrate and of the quantity of *beta* lactose added. Above this point the total initial solubility becomes progressively less than the expected sum. This deviation may result from conversion of some *beta* to *alpha* lactose, since the *beta* lactose is present in amounts greater than its equilibrium concentration.

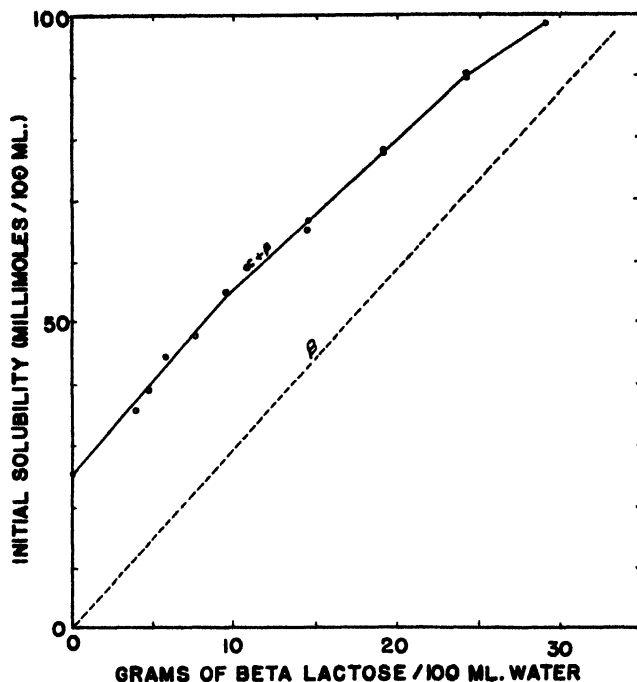


FIG. 1. Standard Curve ($\alpha + \beta$), determined with mixtures containing varying quantities of β -anhydrous lactose and excess amounts of lactose hydrate. Curve " β " plots the amount of *beta* anhydride added.

In table 1 are results showing the effect of sample size. It can be seen that within the range of 10 to 50 g., size of the sample seems to have no effect on the final results. However, with increasing sample size the suspension becomes progressively more viscous and difficult to handle.

To determine the reproducibility of the method, nine separate determinations were made on a sample of nonfat dry milk solids. Results are presented in table 2. Analysis of the results for *beta* lactose calculated as per cent of the total lactose yielded an arithmetic average deviation of 1.03 per cent and a standard deviation of 1.37 per cent indicating fairly good precision.

TABLE 1
Effect of size of sample

Nonfat dry milk solids	Beta lactose	Alpha lactose
(g.)	(%)	(%)
10.0	60.1	39.9
20.0	59.0	41.0
30.0	61.0	39.0
45.0	60.7	39.3
50.0	62.6	37.4

TABLE 2

Reproducibility of the method as applied to a sample of spray nonfat dry milk solids

Trial	Total initial solubility	Beta lactose	Alpha lactose
	(m. mols./100 ml. H_2O)	(%)	(%)
1	51.5	60.0	40.0
2	50.9	58.7	41.3
3	51.2	59.4	40.6
4	50.9	58.7	41.3
5	51.2	59.4	40.6
6	50.1	57.0	43.0
7	52.2	61.5	38.5
8	51.4	59.9	40.1
9	52.2	61.5	38.5
Av.	51.3	59.6	40.4

Arithmetic average deviation = 1.03% beta

Standard deviation = 1.37% beta

In table 3 results are presented for random samples of fresh spray and roller process nonfat dry milk solids and dry whey solids. In dry whey solids samples 1 to 3, the lactose is predominantly in the glass or amorphous state, while in the remaining dry whey samples the lactose is predominantly in the form of the crystalline *alpha* hydrate. The results for nonfat dry milk solids vary from 57.1 to 62.7 per cent *beta* and are in agreement with the values previously reported by Sharp and Doob (7) using the polarimetric method. The nonfat dry milk solids samples and dry whey solids samples 1 to 3 appear to contain *beta* and *alpha* lactose in the equilibrium ratio.

TABLE 3

Alpha and beta lactose content of some dry products of milk calculated as % of total lactose

Sample	Total initial solubility	Total lactose in sample taken	Beta lactose	Alpha lactose
	(m. mols./100 ml. H_2O)	(m. mols.)	(%)	(%)
Spray nonfat dry milk solids				
1	51.4	42.3	61.7	38.3
2	51.4	41.7	62.6	37.4
3	49.5	40.4	59.9	40.1
4	52.4	44.4	61.0	39.0
5	51.3	45.6	57.1	42.9
Roller nonfat dry milk solids				
1 (30.0 g.)	51.6	42.1	62.5	37.5
2	45.8	33.6	61.0	39.0
3	45.5	35.0	57.7	42.3
Dry whey solids				
1	56.4	54.4	57.2	42.8
2	58.7	57.4	58.2	41.8
3	56.9	58.1	54.4	45.6
4	33.3	59.0	13.6	86.4
5	39.8	57.9	25.0	75.0
6	36.4	58.9	18.8	81.2
7	40.4	62.1	24.3	75.7
8	34.0	56.8	15.3	84.7
9	33.5	57.7	14.2	85.8
10	38.0	56.9	22.3	77.7

The method presented in this paper determines total *alpha* and *beta* lactose regardless of the state in which they exist in the dry products. For dry whey solids samples 4 to 10, inclusive, in table 3 most of the lactose has been crystallized as the *alpha* hydrate during processing. The rate of crystallization probably was greater than the rate of conversion of the *beta* to the *alpha* form so that it would be reasonable to believe all *alpha* lactose present in these products to be in the form of the crystalline monohydrate. Therefore, it would be interesting to compare results of the water of crystallization of *alpha* lactose calculated on this basis with those determined by the moisture desorption method and the indirect method previously published (2) for the determination of water of crystallization of *alpha* lactose. Such data are presented in table 4. In general, results by the solubility method are in satisfactory agreement with those by the other two methods.

TABLE 4

Water of crystallization of alpha lactose in some samples of dry whey solids as determined by three different methods

Sample	Water of crystallization		
	Desorption method	Indirect method	Solubility method
	(%)	(%)	(%)
4	2.67	2.75	3.05
5	2.52	2.71	2.61
6	2.89	2.79	2.87
7	2.70	2.76	2.88
8	2.89	2.95	2.81
9	2.89	2.95	2.96
10	2.73	2.62	2.65

SUMMARY

A method has been developed for the determination of the two forms of lactose in dry products of milk based upon the maximum rate of solution of lactose and the difference in solubility of the *alpha* and *beta* modifications. The essential steps consist of (a) adding an excess of *alpha* lactose hydrate to a known quantity of the sample, (b) determining the solubility at several time intervals and (c) extrapolating to zero time to obtain the total initial solubility. The *beta* lactose content may be ascertained easily, since below 55 m.mols. per 100 ml. of water the total initial solubility is the sum of the initial solubility of the *alpha* hydrate and of the quantity of *beta* lactose present. With the *beta* lactose known, the *alpha* modification is calculated by difference from the total lactose.

For a sample of spray process nonfat dry milk solids, it has been found that size of the sample, within the range of 10 to 50 g., seems to have no effect on the results obtained, but with increasing sample size the suspension becomes progressively more viscous and difficult to handle.

The method has been applied to nonfat dry milk solids and dry whey solids.

Results for the nonfat dry milk solids are in agreement with those reported in the literature. For the samples of dry whey solids in which the *alpha* lactose is present as the chief form, water of crystallization has been calculated, assuming complete crystallization of all *alpha* lactose present. Results are in satisfactory agreement with those determined by the moisture desorption method and the indirect method previously published (2) for the estimation of water of crystallization of *alpha* lactose.

REFERENCES

- (1) American Dry Milk Institute, Inc. The Grading of Nonfat Dry Milk Solids and Sanitary and Quality Standards. Chicago, Illinois. 1948.
- (2) CHOI, R. P., O'MALLEY, C. M., AND FAIRBANKS, B. W. Moisture Studies on Dry Products of Milk. II. Estimating Water of Crystallization of Alpha Lactose in Dry Whey Solids. *J. Dairy Sci.*, 31: 619-625. 1948.
- (3) HINTON, C. L., AND MACARA, T. The Determination of Aldose Sugar by Means of Chloramine T with Special Reference to the Analysis of Milk Products. *Analyst*, 52: 668-688. 1927.
- (4) HUDSON, C. S. Hydration of Milk Sugar in Solution. *J. Am. Chem. Soc.*, 26: 1065-1082. 1904.
- (5) HUDSON, C. S. Further Studies on the Forms of Milk Sugar. *J. Am. Chem. Soc.*, 30: 1767-1783. 1908.
- (6) HUNZIKER, O. F., AND NISSEN, B. H. Lactose Solubility and Lactose Crystal Formation. I. Lactose Solubility. *J. Dairy Sci.*, 9: 517-537. 1926.
- (7) SHARP, P. F., AND DOOB, H., JR. Quantitative Determination of Alpha and Beta Lactose in Dried Milk and Dried Whey. *J. Dairy Sci.*, 24: 589-602. 1941.

OBSERVATIONS ON THE APPLICATION OF THE NITROPRUSSIDE TEST TO HEATED MILK

STUART PATTON¹ AND DONALD V. JOSEPHSON¹

Dairy Technology Department, Ohio State University

The nitroprusside test has proven to be a satisfactory technic for studying the presence and behavior of reduced sulfur in various mediums and under widely varying conditions. One of its classic uses is for the qualitative determination of sulfur in organic compounds in conjunction with the sodium fusion reaction (13). Other important applications include its use in studying protein denaturation (1) and in the detection of sulfhydryl compounds (11).

Many sulfur compounds are notorious for their intense and often disagreeable odor. The findings of many workers in the field of dairy research have demonstrated that sulfur compounds are implicated in flavor changes resulting from the heating of milk and dairy products. It is the consensus that consumer acceptance of milk products, the processing of which requires high heat treatment, could be improved greatly if the associated flavor problems could be overcome. The nitroprusside test should be a very useful research tool in the investigation and possible solution of these problems.

REVIEW OF LITERATURE

In 1911 Arnold (2) observed that denatured egg protein gives a color reaction with sodium nitroprusside. Anson (1) since has demonstrated that potassium nitroprusside may be used to measure quantitatively the sulfhydryl groups of denatured egg albumin. Shinohara and Kilpatrick (12) suggest that before hydrogen sulfide is liberated from cystine as a result of heating, the cystine is converted to cysteine, the reduced form. Such a theory is helpful in explaining the mechanism by which sulfhydryl compounds are liberated in heat-denatured proteins, since proteins behaving in such a fashion invariably contain comparatively large quantities of cystine.

Jackson (5) was the first to focus interest on the possible use of the nitroprusside test in connection with milk. He found that raw milk does not give a positive nitroprusside test, but that tests become positive if the milk first is treated with sodium cyanide, a strong reducing agent. He proposed that this phenomenon is traceable to the cystine in the protein complex of milk. Jackson *et al.* (6) reported that sterilization of cream produced "volatile sulfur" which could be detected by the nitroprusside test if heating was carried to 120° C. for 30 minutes. Gould and Sommer (4) could find no correlation between the intensity of the nitroprusside reaction and the temperature at which cooked flavor and sulfide liberation occur in milk. However, they did find some correlation between these factors when sodium cyanide was added to the milk

Received for publication December 11, 1948.

¹ Present address: Department of Dairy Husbandry, Pennsylvania State College, State College, Pa.

following heating. In a similar investigation, Josephson and Doan (7) used the test as a method of measuring sulfhydryl compounds in heated milk and some other heated dairy products. They found very good correlation between the degree of cooked flavor and the intensity of the nitroprusside reaction and concluded that sulfhydryl compounds, liberated during heating, are responsible for cooked flavor of heated milk and milk products. It should be noted that different procedures were followed in the use of the nitroprusside test by these two groups of investigators which might account for the discrepancies in their findings with reference to the test. In fact, Gould (3) in a later publication states that satisfactory results (correlation with cooked flavor) were obtained with the nitroprusside test when a slight modification of the method of Josephson and Doan was utilized. More recently, Townley and Gould (14) used the test with apparent success in extensive experiments dealing with the heat labile sulfides of milk. They noted that prolonged heating of milk at high temperatures results in decreases in the intensity of the nitroprusside test and the quantity of volatile sulfides of the milk. At the same time, a gradual change in the flavor from cooked to caramelized, together with browning of the milk, occurred. Comprehensive data have been gathered on the source of sulfhydryl compounds in milk. The results of investigations by Gould and Sommer (4), Josephson and Doan (7), Gould (3) and Towley and Gould (15) indicate quite conclusively that serum proteins and fat globule membrane protein are the two principal sources.

The foregoing review presents evidence of the usefulness of the nitroprusside test, not only as a means of studying protein denaturation and the presence of sulfhydryl compounds, but also as a suitable instrument for investigating flavor changes resulting from the heating of milk. It seemed worthwhile to determine some of the factors which affect the test as it is applied to milk and to give further consideration to its research possibilities.

EXPERIMENTAL

The procedure of Josephson and Doan (7) for the nitroprusside test was used as a basis for study in these experiments. This procedure is as follows:

"5 ml. of sample were saturated by adding an excess of solid ammonium sulfate in a test tube and shaking. Then five drops of a 4.5 per cent solution of sodium nitroprusside (freshly made) was introduced with agitation, followed by five drops of concentrated (28 per cent) ammonium hydroxide. After again shaking the tube contents, the color was compared with a series of standards. . . ."

According to the observations of various workers (3, 7, 14), the intensity of the pink color developed in the test when it is applied to heated milk appears to be a direct function of the amount of heat treatment to which the milk has been subjected within certain limits. There also is a correlation between the amount of color developed in the test, the quantity of volatile sulfides and the degree of cooked flavor of the milk. Thus, the development of color in the test has quantitative significance in respect to these factors. A visual evaluation of the amount of color produced in the test could be used, but a set of standards made

up with various combinations of dyes provides a more definite comparison for semi-quantitative purposes. Such a set of standards, similar to those used by Josephson and Doan (7), was developed for the present investigation. The following procedure was adopted for making these standards:

Place 10 ml. of skim milk in each of seven test tubes. To each test tube add one drop of 0.1 per cent pontamine brown BT. Then, in successive graduation, add 0, 1, 2, 3, 4, 6, and 8 drops of 0.01 per cent safranin O. Add two drops of chloroform as a preservative to each test tube, seal with rubber stoppers and agitate until the dyes are distributed evenly. Number the standards from 0 to 6, respectively, with increasing concentration of safranin O. Store the standards in a cool place.

Standards made according to the above method give a wide color range and have been found satisfactory for use under most conditions. A possible recommendation is that the 6 standard should contain a small amount of purple color component. Tables contained in this report express intensity of nitroprusside reaction in terms of these numbered standards. The present set of standards is almost a year old and is still in a usable condition.

Since a number of reagents are employed in the nitroprusside test, it was considered advisable to investigate them as potential variables. In these studies raw milk heated to 90° C. momentarily and cooled to 20° C. was used as the test medium. The following quantities or concentrations of reagents were found optimum for the test: sodium nitroprusside, 4.5–5 per cent solution; ammonium hydroxide, not less than 16 per cent strength; ammonium sulfate, 4 to 5 g. per 5 ml. sample. The effect of age of the nitroprusside reagent as a factor in the test also was investigated. Samples of reagent as much as 40 days old gave satisfactory results. It was observed, however, that unless the reagent is stored in the dark, it will impart to the test sample a brown discoloration which intensifies with increasing age of the reagent. One sample of commercial grade ammonium sulfate was found to be unsuitable because of the grey color which it imparted to the test. Generally speaking, it seems advisable to use only the best quality of chemicals for the test.

Previous experience indicated that sample temperature might influence the results obtained with the test as it is applied to heated milk. Experiments concerning this variable showed that temperature is an important factor in the test. Samples of milk which were heated to 95° C. and immediately subjected to the nitroprusside test gave no color development whatsoever. As these samples were cooled to progressively lower temperatures increasing color intensity was secured in the test. The maximum amount of color was obtained when samples were cooled to 20° C. or lower. An appreciable difference in color intensity was produced in samples cooled to 20° as compared with those cooled to 30° C. This effect of temperature appears to be reversible. Samples of milk which were cooled to 20° C., reheated to 60° C. and then subjected to the test developed color comparable to that of samples cooled directly to 60° C.

It was observed further that color stability is enhanced by maintaining the samples at low temperatures during and after subjecting them to the test

TABLE 1

The effect of cooling temperature on permanence of color developed by the nitroprusside reaction in a sample of heated milk^a

Sample treatment	Intensity of NP reaction after holding time (min.) of:						
	0	1	2	3	4	5	30
Cooled to 30° C., tested and held at room temperature (28°) ...	4+	2+	1	±	0	0	0
Cooled to 10° C., tested and held at room temperature (28°) ...	6+	6	5+	5	4	4	1
Cooled to 10° C., tested and held in an ice bath ...	6+	6+	6+	6+	6+	6+	6

^a Heated to 95° C. momentarily and cooled as indicated.

(table 1). Permanence of color is quite advantageous when one wishes to make direct color comparisons between samples. This is accomplished best by cooling the samples in an ice bath, testing them and then storing them in an ice bath. Comparisons should be made within 30 minutes after testing.

The preceding study of the nitroprusside test demonstrated that the procedure of Josephson and Doan (7) for the test is quite satisfactory when temperature is controlled. In the balance of the experimental work reported in this paper, sample temperature was maintained at 20° C. or below for the test.

The disappearance of sulfhydryl compounds in milk under the influence of prolonged high heat treatment raises a new point of interest with respect to their behavior. Townley and Gould (14) were the first to point out this phenomenon and they also noted that it is correlated with browning and caramelized flavor

TABLE 2

The effect of heat treatment on the nitroprusside reaction of skim milk and some of its fractions

Heat treatment	NP reaction			
	Skim milk	Rennet whey	Dialyzed skim milk	Dialyzed skim milk plus lactose ^a
Unheated	0	0	0	0
78.9° C. flash	1	±	1	1
82.2° " "	2	2	2+	2+
87.8° " "	4	5	4	4
93.3° " "	5	6+	5+	5
93.3° 15 min.	6	6+	6	5
93.3° 30 " "	4	6+	4+	3+
93.3° 60 " "	2	6+	4	1+
Color after heating ^b	brown	normal	normal	brown
Flavor after heating ^b	caramelized	cooked	cooked	caramelized

^a 4.76 per cent lactose added prior to heating.

^b 93.3° C. for 60 min.

development in heated milk. In an attempt to clarify this matter, skim milk and some of its fractions were subjected to prolonged heat treatment and the level of sulfhydryl compounds in each fraction was followed during heating by means of the nitroprusside test. After completion of the heat treatment, flavor and color of samples were evaluated by three experienced observers. Table 2 contains representative results from these experiments.

Dialysis of the skim milk was carried out at 4° C. using a cellophane membrane. In other trials it was noted that where the dialyzing water was changed more frequently a higher level of sulfhydryl substances was maintained in the dialyzed skim milk during prolonged heating. The data suggest that the disappearance of sulfhydryl compounds in skim milk heated for prolonged periods is dependent upon the interaction of lactose and casein. This contention is substantiated by data secured from samples of skim milk and its rennet whey after sterilization (116° C. for 15 minutes) and storage (11 months at 37° C.).

TABLE 3

The effect of rennet coagulation on the distribution of sulfhydryl compounds in heated skim milk as measured by the nitroprusside reaction

Heat treatment	Nitroprusside reaction					
	Control skim milk	Whey from heated skim milk	Curd from heated skim milk	Raw Whey	Raw Curd	
Unheated	0	0	0	0	0	
73.9° C. flash	1	0	0	±	0	
82.2° " "	2	±	0	2	0	
87.8° " "	4	±	1	5	0	
93.3° " "	5	±	2	6+	0	
93.3° 15 min.	6	±	2	6+	0	
93.3° 30 " "	4	±	1	6+	0	
93.3° 60 " "	2	±	0	6+	0	

Following sterilization the skim milk had a caramelized flavor and showed considerable evidence of browning. Its reaction to the nitroprusside test was faintly positive. Under these conditions, the whey showed no evidence of caramelization either in flavor or color and gave a very strong reaction to the nitroprusside test. Storage merely amplified these differences. The skim milk became more caramelized and gave a negative nitroprusside reaction, whereas the whey showed a positive reaction and no caramelization after 11 months' storage.

Another interesting property of sulfhydryl substances, with respect to their physical stability, is that they may be precipitated from rennet whey by heat, yet they appear very stable in heated milk. The protein material which is precipitated from whey by heat is quite coarse and gives a very strong nitroprusside reaction. This precipitate has a physical character resembling that of cooked egg white. No such precipitate is obtained by heating skim milk. It would appear that casein prevents the aggregation of serum proteins into large coarse particles in heated skim milk. Keiferle and Gloetzel (8), Matsuo (9) and Menefee *et al.* (10) all have found decreases in albumin nitrogen (in some cases

complete disappearance) and compensating increases in casein nitrogen in heated milk. Matsuo (9) observed that the albumin content remained constant up to 70° C., after which there was a rapid decrease with only a trace remaining above 96° C. These interesting observations prompted an experimental approach using the nitroprusside test as an indicator of sulfhydryl distribution. The disposition of sulfhydryl groups in skim milk heated and then rennet-coagulated was investigated, as well as their disposition in raw skim milk, which was coagulated with rennet and the whey and curd then heated separately. This procedure enabled a study of sulfhydryl distribution to be made both in the presence and absence of casein. In all experiments dealing with this matter it was found that sulfhydryl substances associate themselves with the casein in heated milk. Since these substances have nothing with which to dispose themselves in heated whey, they gather as a precipitate. Data from these experiments are given in table 3. It also was seen that sulfhydryl substances could be removed from heated skim milk only in proportion to the amount of casein removed by supercentrifugation at 35,000 r.p.m. Supercentrifugation of heated whey (95° C. for 30 minutes) deposited a sludge on the centrifuge bowl which gave an intensely positive nitroprusside test.

DISCUSSION

Within fairly wide limits, the strength and the amounts of the reagents used apparently do not influence the sensitivity of the nitroprusside test as it is applied to heated milk. However, sample temperature at the time of testing is a variable which can affect markedly results obtained with the test. When samples of milk are heated to the critical point for sulfhydryl liberation, the intensity of the nitroprusside test will depend upon the temperature at which the test is made. Where temperature is not controlled, it would be possible to obtain negative test results at one temperature and positive results at a lower temperature with any given sample.

It is well to bear in mind that such factors as copper contamination, exposure of the milk to air or sunlight and the amount of lactalbumin present in the milk might influence results obtained with the nitroprusside test when such milk is heated. These factors were not studied in the present investigation.

The complexity of chemical changes taking place in skim milk heated for prolonged periods at high temperatures is revealed by the study dealing with the behavior of sulfhydryl substances in this medium. The data indicate that sulfhydryl substances do not disappear during the prolonged heating of whey, and, theoretically, the same condition obtains with respect to exhaustively dialyzed skim milk. The results which were secured when lactose was added to dialyzed skim milk show the critical importance of this milk constituent in the phenomena of browning, caramelized flavor development and the disappearance of sulfhydryl substances in heated skim milk. The fact that these changes are not observable in heated whey established the importance of casein also in this connection. The logical inference is that sulfhydryl groups disappear by reaction with substances formed from lactose and casein under the influence of heat.

The evidence presented in this and other investigations (8, 9, 10) supports the contention that heat-denatured serum proteins are associated physically and/or chemically with casein in milk heated to high temperatures. Supercentrifugation of heated skim milk should effect a separation of casein and serum proteins containing sulfhydryl groups. Attempts to separate these protein fractions in such a manner were unsuccessful in this investigation. Perhaps the effective mass of the two types of particles is the same, and thus they are removed together, or possibly the two types of particles are associated physically and/or chemically and thus are removed together. The latter presents a more likely explanation. The former, if true, would be quite coincidental. At least, some form of colloidal protective action seems indicated based on the differences in behavior of serum proteins in heated milk as compared with heated whey.

Such an association, resulting from the heating of milk, may be explainable on the basis of changes in electrostatic charge between the two types of protein particles or the liberation of certain chemical groups having an affinity for one another. The differences in curd characteristics of heated and unheated milk may result in part from this association. A casein curd containing denatured serum proteins hardly could have the same physical or chemical characteristics as a pure casein curd. Theoretically, the structure of the curd should be weaker and therefore softer due to the hindering effect of serum proteins on the so-called network formation. The effect of heat in reducing the amount of calcium ion in milk also must be considered as a factor influencing curd tension and curd particle size. In any case, the apparent association of casein and serum proteins in heated milk is worthy of further research.

SUMMARY

The procedure of Josephson and Doan (7) for the nitroprusside test was found to be satisfactory in its application to heated milk when the factor of temperature was controlled. For optimum results, samples should be cooled to 20° C. or lower before subjecting them to the test.

Experiments utilizing the nitroprusside test as an indicator of sulfhydryl behavior demonstrated that these groups disappear in skim milk heated for prolonged periods at high temperatures. This disappearance depends upon a reaction involving lactose and casein and is correlated with the development of caramelized flavor and browning.

The distribution of sulfhydryl substances in heated skim milk was studied, using the nitroprusside test. These substances associate themselves physically and/or chemically with the casein in heated skimmilk. This phenomenon may be an important factor in explaining the soft curd characteristics of highly heated milk.

ACKNOWLEDGMENT

This paper reports research undertaken by The Ohio State University Research Foundation in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned number 232 in the series

of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

REFERENCES

- (1) ANSON, M. L. Some Factors Which Influence the Oxidation of SH Groups. *J. Gen. Physiol.*, **25**: 355-66. 1941.
- (2) ARNOLD, V. Eine Farbenreaktion von Eiweisskörpern mit Nitroprussidnatrium. *Z. Physiol. Chem.*, **70**: 300-309. 1911.
- (3) GOULD, I. A. Cooked Flavor in Milk. A Study of Its Cause and Prevention. *Lab. Sec.*, 33rd Ann. Conv. Intern. Assoc. Milk Dealers. pp. 553-564. 1940.
- (4) GOULD, I. A., AND SOMMER, H. H. Effect of Heat on Milk with Especial Reference to the Cooked Flavor. *Mich. Agr. Expt. Sta. Tech. Bull.*, **164**. 48 pp., illus. 1939.
- (5) JACKSON, C. J. Note on the Sulfhydryl Compounds of Milk. *J. Dairy Research*, **7**, 1: 29. 1936.
- (6) JACKSON, C. J., HOWAT, G. R., AND HOAR, R. P. Discoloration and Corrosion in Canned Cream. Part I. *J. Dairy Research*, **7**, 3: 284. 1937.
- (7) JOSEPHSON, D. V., AND DOAN, F. J. Observations on Cooked Flavor in Milk: Its Sources and Significance. *Milk Dealer*, **29**: 35-41. 1939.
- (8) KEIFERLE, W., AND GLOETZEL, J. Veränderungen der Milch, in *Handbuch der Milchwirtschaft*, edited by Winkler, published by Springer, Vienna. **1**, 1: 231. 1930.
- (9) MATSUO, I. Biochemical Study of Milk. IV. The Effect of Heating on the Constituents of Milk. *Osaka, J. Med.*, **28**: 555-562. 1929. (Cited from *Chem. Abs.*, **24**: 1160. 1930.)
- (10) MENEFFEE, S. G., OVERMAN, O. R., AND TRACY, P. H. The Effect of Processing on Nitrogen Distribution in Milk. *J. Dairy Sci.*, **24**, 11: 953-968. 1941.
- (11) MULLIKEN, S. P. *Identification of Pure Organic Compounds*. John Wiley and Sons, New York. Vol. IV, p. 17. 1922.
- (12) SHINOHARA, K., AND KILPATRICK, M. The Stability of Cystine in Acid Solution. *J. Biol. Chem.*, **105**, 2: 241-51. 1934.
- (13) SHRINER, R. L., AND FUSON, R. C. *The Systematic Identification of Organic Compounds*. 3rd ed. John Wiley and Sons, New York. P. 54. 1948.
- (14) TOWNLEY, R. C., AND GOULD, I. A. A Quantitative Study of Heat Labile Sulfides of Milk. I. Method of Determination and Influence of Temperature and Time. *J. Dairy Sci.*, **26**, 8: 689-703. 1943.
- (15) TOWNLEY, R. C., AND GOULD, I. A. A Quantitative Study of Heat Labile Sulfides of Milk. II. General Origin of Sulfides and Relation to Total Sulfur, Total Nitrogen and Albumin Nitrogen. *J. Dairy Sci.*, **26**, 9: 843-851. 1943.

MILK SURFACES. I. THE SURFACE TENSION OF FRESH SURFACES OF MILK AND CERTAIN DERIVATIVES¹

CARRELL H. WHITNAH,² RALPH M. CONRAD,³ AND GLEN L. COOK⁴

This investigation started with the assumptions that the surface tension of a freshly-formed milk surface should not differ greatly from the surface tension of pure water and that it might be possible to observe early changes in surface tension of freshly-formed milk surfaces. At least, it was hoped to find ways of so modifying or fractionating milk that an early rapid fall in surface tension could be measured. Any such fall in surface tension would be some indication of the accumulation or orientation of surface-active material on the surface.

REVIEW OF LITERATURE

A sufficient introduction to the history of measurements of the surface tension of milk and to the nature of surface-active substances to be expected in milk is given in recent papers by El-Rafey and Richardson (5, 12) and by Aschaffenburg (3) and in papers cited by these authors.

The vibrating-jet method of measuring surface tension as proposed by Rayleigh (11) and refined by Pedersen (10) and Bohr (4) seemed most promising for very young surface ages. This method was used with some simplification by Addison (1) to measure the rate of fall of surface tension of solutions of certain alcohols. In Addison's solutions the fall of surface tension measured a rate of adsorption in the surface from the bulk of the solution. Addison referred to the changing surface tension in young surfaces as dynamic, and to the constant value obtained later as static. He considered his static values to represent a true equilibrium between the surface and the bulk of the solution. If a surface-denatured protein accumulates on a milk surface and if this denaturation is irreversible, the static or nearly constant surface tension would not represent a true equilibrium (8). The simplified theory of this method is that if a jet of liquid flows from an elliptical orifice, the surface tension of the jet pulls it into circular cross section. The momentum of this motion later produces another elliptical cross section with the major axis at right angles to the major axis of the orifice. Repetition of this process produces a series of standing waves which can be photographed and measured. With any given set of conditions, a decrease of surface tension increases the wave length.

Received for publication December 17, 1948.

¹ This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned no. 235 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of the Army. The paper contains material presented by Glen L. Cook to the University of Denver as partial fulfillment of the requirements for a Master of Science degree in chemistry.

² Present address Chemistry Department, Kansas State College, Manhattan, Kansas.

³ Deceased.

⁴ Present address U. S. Bureau of Mines, Laramie, Wyoming.

The formulas of Pedersen and Bohr (10, 4) using suitable symbols may be expressed as follows:

$$T = \frac{(P_1 + P)k^2 r^3 c^2 J_2(irk)}{(3 + r^2 k^2)irk J_2'(irk)} \times \left[1 + 2 \left(\frac{2\mu}{Per^2 k} \right)^{3/2} 3 \left(\frac{2\mu}{Per^2 k} \right)^2 \right] \times \left(1 + \frac{37}{24} \frac{n^2}{m^2} \right)$$

T = Surface tension dynes/cm.

P_1 = Density of medium (air) g./cc.

P = Density of liquid g./cc.

$k = 2\pi/\lambda$. $\pi = 3.1316$.

l = Wave length cm. (node-to-node or loop-to-loop).

r = Equivalent radius or \sqrt{ab} .

$a = 1/2$ long axis = "r max."

$b = 1/2$ short axis = "r min."

c = Velocity of jet cm./sec. = $f/(\pi ab)$.

f = Flow rate of jet g./sec.

μ = Coefficient of viscosity.

$m = (a + b)/2$. $n = (a - b)/2$. $i = \sqrt{-1}$.

J_2 = Bessel's function of order 2.

J_2' = First derivative of J_2 .

$$u_2 = 4\sqrt{\pi} \cdot \frac{J_2(irk)}{(3 + r^2 k^2)irk J_2'(irk)} \quad (\text{Given in Pedersen's table for values of } rk.)$$

The term in brackets is Bohr's correction for the viscosity of the liquid in the jet. The third term $\left(1 + \frac{37}{24} \cdot \frac{n^2}{m^2} \right)$ is Bohr's correction for the finite difference $a - b$. A finite value of this difference definitely was excluded in Rayleigh's original calculations but is needed to make the wave lengths measurable. These last two terms were omitted in Pedersen's formula. They are not important in the work of Pedersen or Bohr, but in the present study were found to reach values as high as 1.02 and 1.20, respectively.

After years of study with other methods of measuring surface tension, Harkins and Anderson (6) selected the Wilhelmy hanging slide for measuring differences in static surface tension. Materials for a similar apparatus were available for parts of the present study. The pendant drop method of Andreas *et al.* (2) might have been more suitable but would have required the building of another elaborate apparatus.

Although there are no known studies of possible decomposition products arising from the method of removing protein fractions proposed by Harland and Ashworth (7), this treatment seemed mild and was different from the methods used by El-Rafey and Richardson (5) and by Aschaffenburg (3).

APPARATUS

The optical methods for measuring the wave lengths of vibrating jets that depend on the lens-like action of the jet are not available for an opaque liquid like milk. Therefore, the jets were photographed and measurements of both length and diameter were made from these photographs. On early plates both the lengths and diameters were measured on a projected image of the photograph where the total magnification was about 25. On later plates the diame-

ters were measured with a microscope fitted with a mechanical stage capable of reading .0001 cm. This stage was mounted on a lathe carriage so that the longitudinal and transverse feed screws measured changes in position of the plate. Rotation of the arm carrying the microscope permitted the selection of a convenient range for the cross feed. A mirror from a 16 mm. gun camera permitted a horizontal eyepiece and also permitted alignment of the image of the jet axis with the longitudinal motion of the stage.

The position of the wave ends on the photograph could not be judged as accurately with the microscope as on the screen. Therefore, all wave lengths were measured on the screen. In early photographs the pictures were taken with white light. Later a mercury vapor lamp with Corning filters to isolate the line at 435 $m\mu$ was used.

Orifices were arranged to produce three simultaneous, nearly horizontal jets in a single vertical plane. The axis of a machine screw, selected for uniformity of thread pitch, also was placed in this plane to serve as a basis for determining

TABLE 1
Characteristics of Orifices

Orifice no.	Material	Major axis	Minor axis	Thickness	Surface tension of water at 25° C.
		(cm.)	(cm.)	(cm.)	(dynes/cm.)
1	Steel	.050	.035	.0051	73.9
2	Brass	.084	.069	"	72.3
3	Steel	.127	.100	"	71.1
4	Glass	.0424	.0318	.0170	95.3
5	"	.0645	.0466	"	61.6
6	"	.0724	.0636	"	72.3
7	Gold	.0422	.0304	.0045	71.6
8	"	.0503	.0369	"	71.0
9	"	.0737	.0540	"	70.2

magnification. The three simultaneous pictures helped to separate irregularities in the jets and their measurements from variation between samples of milk.

The orifices were formed in thin plates because of Addison's finding (1) that initial disturbances in the jets vanish more quickly if the orifices are plates rather than tubes. Three groups of orifices were tried.

In the first group, an orifice was formed in a thin sheet of brass or steel. To form an orifice, a round hole was first drilled in a plate with a jeweler's drill of the size desired for the minor axis. The hole then was made nearly elliptical by tilting the drill. The final shape of the hole then was formed by filing with a roughened wire until the shape appeared satisfactory under a microscope. A steel orifice was of harder material and could be fashioned more easily into acceptable shape but rusted easily so that repolishing was necessary before each use. It was difficult to obtain a smooth edge on a brass orifice as the sheet showed grain, fiber or flaws. The surface tension of milk at ages from .0007 to .0265 seconds was measured with these orifices, using white light and the projector for both the lengths and diameters of the waves. Some

characteristics of these orifices are given as numbers 1, 2 and 3 of table 1. The sheet was soldered onto the end of a brass tube and the tube was mounted with glyptal cement in the end of a glass tube. This procedure of mounting was satisfactory if the orifices could be aligned by inspection. When the photographs were twice natural size it was necessary to produce jets and align the orifices before the cement hardened completely. After such procedure some cement dissolved and frequently accumulated in the orifice, changing its size and shape.

The second group of orifices was made in glass plates. An electric spark was used first to punch or tear a hole in a pyrex coverglass made for microscope slides. This hole then was enlarged with a soft wire carrying an abrasive. The hole was finished much as in the first system. It was not possible to get the edges of the orifice entirely free from splinters extending part way through the thickness of the glass. Also, the edge near the ends of the major axis was beveled somewhat. Three orifices of this type were cemented to the ends of glass tubes and baked in an oven to harden the cement. The tubes then were sealed to the supply tubes in such a way that the orifices appeared in proper position. The tubes were realigned, if necessary, to bring the three jets into one plane. It was not found possible to make the three jets exactly parallel in this plane. Jets from these orifices were reproducible and could be read for a greater number of waves than for orifices of the first system. However, the jets were not satisfactory because the surface tension of water was irregular and because of uneven wave lengths and of irregular positions of occasional nodes. These orifices are described as numbers 4, 5 and 6 of table 1.

The third group of orifices was made in sheets of 14-karat gold. Three tapered holes were drilled on the center line of a stainless steel plate which fitted as a cover for one side of a tank or chest of similar metal. A sheet of gold then was soldered over the small end of each hole and the entire assembly imbedded in plastic. The three orifices then were drilled through the plastic and the gold plates with a Gortzen pantograph milling machine. The plastic was removed after the orifices were formed. The controlled motion of the table of the milling machine permitted accurate placing of both the position and direction of the axes of the orifices. These orifices are numbers 7, 8 and 9 of table 1.

The orifices, the machine screw and nearly all of each jet were enclosed in a jet house. This was to avoid evaporation from the surface of the jets, change in temperature of the jets and disturbance from currents of air. The jet house had double windows on both the side toward the condensing lens and on the side toward the camera. Just enough warm air was blown into the space between each of these double windows to prevent fog from collecting on the inner surfaces. Three holes in the end of the jet house away from the orifices permitted each of the three jets to flow into the container or jet catcher where the flow for a measured time was caught and weighed. The three containers were mounted in a rack which could be moved along sliding ways in front of the jets as a stopwatch was started and away from the jets as the watch was stopped. A diagram of the final form of the apparatus is shown in figure 1.

For static measurements of surface tension, a torsion balance with dial calibrated to read 500 mg. at 2 mg. per division was used. A table on a vertical screw was mounted under the balance arm. Rods from this table extended down through the base of the balance into an air-conditioned metal cabinet where they carried a shelf on which samples were placed. A fine wire extended from the

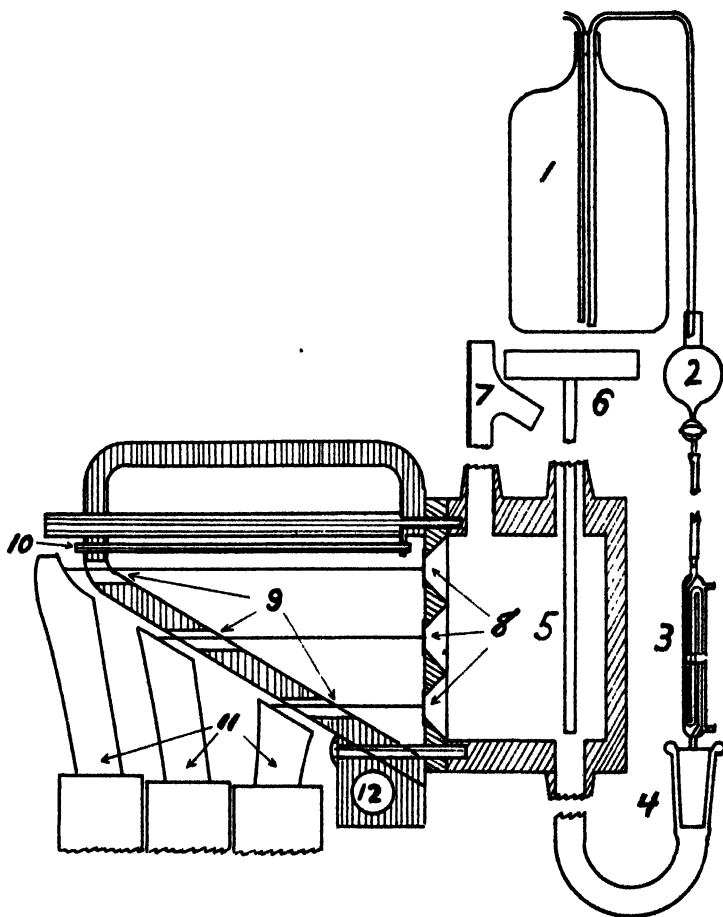


FIG. 1. Vibrating Jet Assembly Parts 1-3 = $\frac{1}{8}$ size. Parts 4-12 = $\frac{1}{4}$ size. 1. Supply with syphon and control. 2. Reservoir with stopcock. 3. Heat exchanger. 4. Ground glass joint. 5. Stainless steel chest. 6. Dial thermometer in glass joint. 7. Overflow tube. 8. Tapered holes, orifices, and jets. 9. Exit holes in jet house. 10. Machine screw for scale. 11. Jet catchers and containers. 12. Inlet for warm air. Steel section = ||| = |||. Brass section = ||| = =.

balance arm into the cabinet to carry the slide which made the surface contact. The slides used were 22×35 mm. pyrex coverglasses made to cover microscopic slides. A lamp outside, but near a window of the cabinet, kept the window free from condensed moisture and also lighted the interior of the cabinet.

When milk was rehomogenized, this was done by passing it three times through a single-cylinder hand homogenizer.

PROCEDURE

To measure surface tension by the vibrating-jet method, milk or other liquid, cooled below the lowest temperature to be used, was placed in the supply system. Water to maintain the desired temperature was placed in or circulated through the outer chamber of the heat exchanger. The liquid was allowed to flow at a rate to supply the jets and cause from 30 to 120 drops per minute to fall from the overflow.

While the chest and jet house were coming to temperature equilibrium, the alignment and focus of the camera and lamp were checked. If the experiment was being conducted at more than 5° C. above room temperature, a stream of warm air was blown through the space between the double windows of the jet house. As soon as the temperature in the chest became constant, the weighed jet-catchers were slid in front of the jets with one hand while a stop watch was started with the other. After about 1 minute the air-blower and water-circulating pump were stopped for a few seconds to avoid vibration while the actual photographic exposure was being made. The pump then was restarted and let run until the discharge from the jets had been collected for about 2 minutes. The jet catchers then were pushed away from the jets and the watch was stopped. The circulating pump, water bath heater and lamp then either were turned off or were set for a higher temperature.

The jet-catchers containing the liquid collected during the measured time were wiped clean, adjusted to room temperature and weighed. The temperature at the time of exposure and the reading of the stop-watch were recorded.

Wave lengths were measured by projecting the photograph of the jet on a screen of drawing paper. The position of each node and loop was marked on the screen. Then the distance of each node ($N_0, N_1, N_2 \dots$) and each loop ($L_0, L_1, L_2 \dots$) from the orifice was measured. The marking and measuring of these positions were repeated ten times and the ten values for each distance were averaged. The difference between successive average values of N or L was taken as a wave length (1). The wave beginning at N_0 and ending at N_1 was considered to have an average distance from the orifice of L_0 . This distance and the flow rate were used to calculate the age of this wave.

If diameters were measured on the screen, the procedure was similar to that for wave lengths. If diameters were measured with the microscope, the plate was placed on the stage so that the axis of the jet would remain on a center line in the eyepiece during longitudinal motion of the carriage. The longitudinal position for the first minimum width (N_0) of the jet was selected and the width measured ten times. These values then were averaged for ND_0 . Similarly for LD_0, ND_1 , etc.

The diameter measured at L_0 was used as the major axis (2a) there. The corresponding minor axis (2b) was assumed to be the average of values measured at N_0 and N_1 . When the correction factor for amplitude was not used,

$2a$ and $2b$ were taken as the average of all available consecutive values, with the limitation that first and last values were either both nodes or both loops. These average values were used to determine the equivalent radius (r) even when the particular correction factor for amplitude was applied to each wave.

A specific gravity balance and an Ostwald viscosimeter were used to determine the densities and viscosities at the temperatures used for measuring surface tension. The values were determined relative to water (9).

For measurements with orifices 1-3 the first term of the formula of Bohr (4) and Pedersen (10), as given above, was used without either correction factor. For measurements with orifices 7-9, the amplitude correction was used on each wave. The viscosity correction was important only for evaporated milk.

The liquids thus tested included (a) water, (b) commercially homogenized milk, (c) evaporated canned milk, (d) rehomogenized milk diluted 10 times with water, (e) the filtered serum obtained after saturating milk with sodium chloride and holding overnight at 40° C., and (f) the above serum after bringing to pH 2 with hydrochloric acid, holding overnight and filtering again. Milk and the two sera just described also were diluted with water until changes of surface tension with surface age could be measured by the jet method and also, after further dilution, on the static balance.

RESULTS AND DISCUSSION

The surface tensions of water measured with the orifices used for milk and other liquids are shown in the last column of table 1.

A summary of 215 measurements on commercial homogenized milk at 25° C. and at surface ages ranging from .0007 to .0265 seconds, is shown together with standard deviations (13) in table 2. There was some decrease of surface tension within this range of surface age. However, the surface tension of the youngest surface was still nearly equal to the static value for milk.

The gold orifices produced longer jets from each of which a greater number of waves could be measured. Also overtones were not noticeable in these jets. The values for surface tension at different ages, however, were no more consistent than values in table 2 for the same surface ages. The measurements on milk with the gold orifices therefore were used only to detect any change of surface tension at the youngest possible ages. The average correction factors needed to reduce the surface tension of water and of milk at L_0 and N_1 to the average value from L_1 to the end of the jet (x) are shown in table 3.

At L_0 the factors for milk are only slightly larger than corresponding factors for water, for orifices 7 and 8. For orifice 9 the factor for milk is slightly smaller than for water. If the surface tension of milk relative to water were decreasing at these surface ages, the correction factor for milk should be correspondingly larger than for water. The similarity of factors indicates that there was no great change in the surface tension of milk at these surface ages. Therefore, most of any change in the surface tension of milk relative to water had taken place before the surface had reached the age of .0003 seconds.

It is not surprising after the above results that no change with age was

TABLE 2

Summary of surface tensions of commercially homogenized milk at 25° C. and increasing surface ages

No. of values averaged	Av. age	Surface tension (dynes/cm.)		
		Av.	Standard deviation	Standard error of mean
	(sec.)			
7	.0007	58.39	2.52	.95
17	.0014	56.07	2.42	.59
14	.0021	55.08	2.90	.77
19	.0028	55.28	4.01	.92
19	.0035	55.10	4.66	1.07
9	.0042	53.29	2.12	.71
19	.0049	50.51	3.16	.72
10	.0056	52.48	2.27	.72
8	.0063	50.98	1.97	.70
11	.0070	50.45	3.11	.94
11	.0080	51.38	2.86	.86
11	.0090	51.42	2.36	.71
8	.0100	52.05	2.88	1.02
5	.0110	52.11	1.56	.70
9	.0120	52.60	2.67	.89
10	.0130	50.45	2.46	.74
8	.0145	50.30	4.89	1.73
7	.0165	49.51	2.52	.95
5	.0185	49.00	2.03	.91
3	.0205	51.96	2.21	1.28
3	.0225	52.07	2.93	1.69
2	.0265	46.82	1.73	1.22

found in the surface tension of evaporated milk. The viscosity of .199 poises of this product at 15° C. was so great that wave lengths in the jets could not be measured, although a very clear picture of the jet was obtained. Measurements at 48 and 66° C. gave average surface tensions of 50.3 and 50.7 dynes/cm., respectively. Viscosities at these temperatures were .045 and .024 poises, respectively.

When milk was modified by diluting with 10 parts of water or by removing the casein or total protein (7) the surface tension did change at measureable values of surface age. From varying short intervals after the first measurements, the surface tension dropped at a rapid, nearly constant rate. After a later surface age, which also varied with the particular liquid, the surface tension changed at

TABLE 3

Correction factors for surface tension at L_0 and N_1 needed to make these values equal to corresponding average values for L_1 to end of the jet (x)

Orifice no. Wave no.	7			8			9		
	L_0	N_1	x	L_0	N_1	x	L_0	N_1	x
Liquid	Correction factor								
Water	.7610	.9634	N_7	.7657	.9452	L_8	.9416	.9560	N_9
Milk	.7987	.9124	N_8	.8026	.9363	N_8	.8992	.9569	N_9
Surface age (sec. $\times 100,000$)	28	55		45	80		82	133	

TABLE 4
Surface tension of modified milks at characteristic surface ages

Liquid	Milk diluted 1 milk + 10 water				Non-casein filtrate		Non-protein filtrate	
Temp. ° C.	50		15		15		15	
Characteristic	Age ¹	T ²	Age	T	Age	T	Age	T
Fall starts	10	65.8	20	66.0	10	88.0	10	91.0
Break ³	20	61.0	60	59.0	35	80.0	30	80.0
1st rate ⁴	4800		1750		3200		5500	
Oldest age	70	55.4	100	57.0	70	79.4	70	79.2
2d rate	1120		500		171		200	
Static T	44.0		50.4		54.7		51.0	

¹ Seconds $\times 10,000$.

² Surface tension (*dynes/cm.*)

³ Intersection of two nearly straight portions of curve for surface tension vs. surface age.

⁴ Rate of fall in surface tension (*dynes/cm./sec.*).

another nearly constant but much slower rate (table 4). Before using these data as precise characteristics of the modified milks, many more observations and more careful definitions of the liquids are needed. The data, however, do furnish a definite contrast to the nearly constant surface tension of whole milk at similar surface ages.

A 1 per cent solution of milk, or either of the above filtrates undiluted, would reach a constant surface tension within 1 minute. Dilutions of 1 per cent, for the filtrate, or of .01 or .005 per cent for milk were satisfactory for observing, with the static balance, the change in surface tension. Static surface tensions of rehomogenized milk and of commercially-homogenized milk, freshly warmed to temperatures from 15 to 70° C. after storage at about 5° C. for 1 to 7 days, and of fresh dilutions of the stored commercially-homogenized milk are shown in table 5. For each pair of values at a given age and temperature, the surface tension for the commercially homogenized milk was slightly greater than for the rehomogenized milk. Data are not available to show how universal this relationship is.

TABLE 5

Static surface tensions of rehomogenized milk and commercially homogenized milk freshly warmed to indicated temperatures after storage at about 5° C. for 1 or 7 d. and of fresh dilutions of the stored commercially homogenized milk

Temp. ° C.	Rehomogenized		Commercial		Commercial diluted to:			
	1 d.	7 d.	1 d.	7 d.	10%	1%	.1%	.01%
(dynes/cm.)								
15	47.9	46.1	53.5	48.0	50.4	...	53.3	.
25	46.3	45.0	48.8	45.9	46.9	51.6	51.5	56.5
30	45.5	44.8	46.7	45.4	45.8	50.2	50.2	53.8
40	44.9	43.9	45.1	44.9	44.8	49.2	47.4	52.7
50	43.3	43.3	44.2	43.7	44.0	48.0	46.4	51.9
60	42.8	42.6	44.0	44.1	43.6	46.9	45.6	50.9
70	41.8	43.0	43.5	...	42.7	46.5	43.9	45.9

TABLE 6

Surface tensions of diluted milk, diluted non-casein filtrate and non-protein filtrate in relation to cleaning of slide before each reading, depth of solution and surface age

Temp. ° C.	25	25	20	20	20	20
Cleaned slide	No	No	Yes	Yes	Yes	No
Substance	Milk	Milk	Milk	N.C.F.	N.P.F.	N.P.F.
Diln. to %	.005	.005	.01	1	1	1
Vol. ml.	20	120	20	20	20	20
Container	A	A	B	B	B	B

Age (min.)	Surface tension (dynes/cm.)					
1	71.0	70.5	72.1		72.6	71.7
1.5	70.5	69.7	71.6	55.0		71.4
2	70.1	69.0		54.7	72.6	71.0
3	69.0	67.6	70.8	54.5		70.5
4	68.8	66.8				69.9
5	68.3	66.0	70.8	54.3		69.2
8	67.9	64.8	70.8	54.1	72.5	67.9
12	67.4	63.9	70.5	53.8	72.5	66.3
18	67.4	62.9	69.4	53.6	73.1	64.3
73				53.0		
93			63.2			
115				52.7	72.2	
176			60.4			
225			60.4	52.3	70.4	
346					64.9	
390					62.5	
626					61.8	
1146				50.3		
1338					59.6	

In table 6 are shown surface tensions of dilutions of milk, non-casein filtrate and non-protein filtrate, measured with the static balance with and without cleaning the cover glass before each measurement, on solutions of different depths and at different surface ages.

TABLE 7

Rate of fall in surface tension, at various ages, of high dilutions of each milk, non-casein filtrate, and non-protein filtrate; from values in table 6

Surface Age (min.)	Rate of fall (dynes/cm./min.)		
	.01% milk	1% N.C.F.	1% N.P.F.
1.5	1.0		
2		.6	
12	.08	.08	.00
93	.08		
115			.003
176	.046		
225	.000	.036	.016
346			.045
390			.055
626			.003
1146		.002	
1328			.003
Total fall as % of in- itial value	16.2	8.5	17.9

The progressively smaller fall in the surface tension of the 20 ml. sample of .005 per cent milk compared with the 120 ml. sample at identical surface ages indicates that the smaller sample contained an inadequate amount of surface active material to quickly or fully saturate the surfaces of the solution.

For the two identical solutions of non-protein filtrate, the consistently lower values with the slide not cleaned before each measurement (table 6) indicate that the contact edge or surface of the cover glass must absorb an appreciable amount of surface active material before it can give a constant tension. It seems quite possible that this absorption may produce a contact angle greater than zero. Such a disturbance probably would be detected less easily but be more serious with a wire ring than with the cover glass.

The changes of surface tension with age when the cover glass was cleaned before each measurement and when the volume of the solution and the shape of the container were identical are shown in table 7.

The initial objective of this phase of the study was to modify milk so that the rate of change of surface tension could be observed with simple apparatus. This objective has been achieved. An explanation of the lag of nearly 2 hours before the surface tension of the diluted non-protein filtrate started to fall or of the relatively small total fall in the surface tension of the non-casein filtrate is outside the scope of this paper.

SUMMARY

The vibrating-jet method of measuring surface tension was applied to milk.

The surface tensions of commercially homogenized milk at 25° C. were determined at surface ages ranging from .0007 to .0265 seconds. Correction factors needed to reduce the measured surface tensions at surface ages as young as .0003 seconds to average values excluding the first two waves were nearly identical for milk and water. Any great change in the surface tension of milk relative to water therefore must have taken place before the surface age of .0003 seconds.

When milk was diluted with ten parts of water, or when the casein was precipitated by saturating the milk with sodium chloride or when other proteins also were precipitated by acidifying with hydrochloric acid, large decreases of surface tension were found in the age range .001 to .01 seconds.

When milk was diluted to .01 per cent or when the above filtrates were diluted to 1 per cent, decreases in surface tension could be observed by ordinary methods for periods of several hours.

REFERENCES

- (1) ADDISON, C. C. The Measurement of Surface and Interfacial Tensions at Fresh Surfaces by the Vibrating Jet Method. *Phil. Mag.*, **36**: 73-100. 1945.
- (2) ANDREAS, J. M., HAUSER, E. A., AND TUCKER, W. B. Boundary Tension by Pendant Drops. *J. Phys. Chem.*, **42**: 1001-1019. 1938.
- (3) ASCHAFFENBURG, R. Surface Activity and Proteins of Milk. *J. Dairy Research*, **14**: 316-329. 1946.
- (4) BOHR, NIELS. Determination of the Surface Tension of Water by the Method of Jet Vibration. *Roy. Soc. London, Phil. Trans.*, **A209**: 281-317. 1909.
- (5) EL-RAFEY, M. S., AND RICHARDSON, G. A. The Role of Surface-active Constituents

- Involved in the Foaming of Milk and Certain Milk Products. I and II. *J. Dairy Sci.*, 27: 1-31. 1944.
- (6) HARKINS, W. D., AND ANDERSON, THOMAS F. A Simple Accurate Film Balance of the Vertical Type for Biological and Chemical Work. *J. Am. Chem. Soc.*, 59: 2189-2197. 1937.
 - (7) HARLAND, H. A., AND ASHWORTH, U. S. Preparation and Effect of Heat Treatment on the Whey Proteins of Milk. *J. Dairy Sci.*, 28: 879-896. 1945.
 - (8) HAUSER, E. A., AND SWEARINGEN, L. E. The Aging of Surfaces of Aqueous Solutions of Egg Albumin. *J. Phys. Chem.*, 45: 644-659. 1941.
 - (9) HODGMAN, CHARLES D. Handbook of Chemistry and Physics. 19th ed. Pp. 1115, 1142. Chemical Rubber Pub. Co., Cleveland, Ohio. 1934.
 - (10) PEDERSEN, P. O. Surface Tension of Liquids Investigated by the Method of Jet Vibrations. *Roy. Soc. London, Phil. Trans.*, A207: 341-392. 1907.
 - (11) RAYLEIGH, LORD. On the Capillary Phenomena of Jets. *Proc. Roy. Soc. (London)*, 29: 71-97. 1879.
 - (12) RICHARDSON, G. A., AND EL-RAFEY, M. S. The Role of Surface-active Constituents Involved in the Foaming of Milk and Certain Milk Products. III. *J. Dairy Sci.*, 31: 223-239. 1948.
 - (13) SNEDECOR, GEORGE W. Statistical Methods. 4th Ed. Pp. 36, 61. Iowa State College Press. Ames. 1946.

EFFECTS OF VITAMIN A AND CAROTENE INTAKE ON DEPLETION TIME OF YOUNG DAIRY CALVES

W. C. JACOBSON, H. T. CONVERSE, AND L. A. MOORE

*Bureau of Dairy Industry, Agricultural Research Administration,
United States Department of Agriculture*

The present practices of rearing dairy calves with limited amounts of whole milk have altered considerably the quality and quantity of food a calf receives up to 4 months of age, as compared with former feeding practices in which whole milk was fed more liberally or the calves were allowed to suckle their dams. Insofar as vitamins are concerned, the intake of vitamin A has been reduced materially by present-day feeding practices. Moore and Berry (8) found that when calves were reared according to present-day methods of limited whole-milk feeding, the vitamin A content of the blood plasma from birth to 4 months of age was in the deficient range, as judged by blood values of vitamin A-deficient calves 4 to 14 months of age. Moore *et al.* (9) reported that the vitamin A content of the blood plasma of dairy calves reared on a limited whole-milk feeding program was one-third lower than that of beef calves of the same age that were permitted to suckle and thus obtained considerably more whole milk. Wise *et al.* (13) found that the vitamin A content in the blood serum of calves at 5 weeks of age was 50 per cent lower than it was at the colostrum feeding period and at 10 weeks of age when the calves had started to consume more hay.

Krauss *et al.* (5) reported a decrease in the incidence of pneumonia in calves that received 15,000 I.U. of vitamin A concentrate daily as compared with a similar group that received no concentrate. Gullickson and Fitch (3) noted less trouble from digestive disturbances in young calves fed cod-liver oil than in calves not receiving the supplement. Whole milk was fed at the rate of one-eighth of the body weight per day for the first 30 days, followed by skim milk to 6 months of age. While calves in both groups had scours, some of the calves that received no supplement died. Phillips *et al.* (12) reported that the administration of a high vitamin A potency shark-liver oil plus certain of the B vitamins eliminated diarrhea and lowered the mortality caused by pneumonia.

In experiments at the Michigan station Moore (7) found that young calves invariably died of pneumonia and scours before 3 months of age when placed on a vitamin-A deficient ration. Converse and Meigs (1) showed that calves on a low vitamin-A intake died before 100 days of age. These reports suggest that vitamin A might have played a part in building resistance against bacterial infections in young calves. The evidence, while not clear-cut, seems to indicate that calves reared according to present-day methods of limited whole milk feeding may not receive sufficient vitamin A from birth to 4 months of age.

A summary of the Beltsville data (2) on the vitamin A requirements of calves from birth to 6 months of age indicates that a minimum daily intake of between

Received for publication December 20, 1948.

10 and 25 γ of vitamin A (from cod-liver oil) per lb. of body weight was necessary to maintain normal growth. A study of the data reported by Lewis and Wilson (6) indicates that the minimum requirement for growth lies between 4.5 and 9 γ of vitamin A daily per lb. of body weight. These figures are somewhat lower than those reported from Beltsville. However, Lewis and Wilson (6) found that a daily intake of 9 to 18 γ of vitamin A per lb. of body weight was necessary to maintain the blood plasma vitamin A values above the deficient range.

Apparently the minimum quantity of vitamin A required per lb. of body weight to maintain good health, growth, and a normal level of vitamin A in the blood lies between 18 and 25 γ per day per lb. of body weight. If an arbitrary value of 25 γ is chosen and this figure is doubled for optimum results under practical farm conditions, a 100-lb. calf would need 5,000 γ of vitamin A or about 20,000 I.U. per day.

In 1941 Phillips *et al.* (12) noted that supplementing the ration with 5,000 I.U. of vitamin A plus niacin and ascorbic acid in capsules was effective in preventing scours and pneumonia in young calves. These data have received wide publicity, and as a result, several drug houses have placed capsules on the market containing these ingredients. However, other data (4, 11) show that the feeding of these capsules is of little value in preventing scours in calves reared under practical farm conditions. Possibly addition of 5,000 I.U. per day to the ration was not sufficient to bring out differences in response of calves to vitamin A feeding.

Apparently there is need for further carefully controlled experiments to determine the requirements for vitamin A and also the value of vitamin A supplements in the ration of the dairy calf up to 4 months of age, especially the effect of supplementing with larger quantities than 5,000 to 10,000 I.U. per day.

The object of this experiment was to determine the difference in blood plasma vitamin A levels and vitamin A stores between calves fed various amounts of supplemental vitamin A and those reared under natural conditions, as well as those reared using present-day feeding practices.

EXPERIMENTAL PROCEDURE

Fifty-two calves of the Jersey and the Holstein breeds were used in this experiment. Since no data are available on the blood plasma vitamin A and carotene levels in dairy calves reared under natural conditions, two cows were turned on pasture approximately 2 weeks before parturition. The cows calved on pasture and the calves were allowed to run with their dams on pasture until they were 90 days of age. Thus the calves not only had access to all the whole milk they could consume, which would be high in vitamin A, but they also had access to whatever fresh grass they desired to consume.

Fifty male calves received colostrum for 3 days and then were given a limited quantity of whole milk daily to 60 days of age and a grain mixture and alfalfa hay from birth to 90 days of age. A total of about 360 lb. of whole milk was fed to 60 days of age in this experiment. Twenty-four of these calves received sup-

plementary vitamin A in cod-liver oil. Eleven of the 24 calves received 25,000 I.U. of vitamin A per day for varying periods of time up to 42 days of age, and 13 of the 24 received 50,000 I.U. of vitamin A per day to various periods ranging up to 90 days of age. Ten to 15 ml. of cod-liver oil were used to furnish 25,000 I.U. of vitamin A, depending on its potency, while double these quantities was used to furnish 50,000 I.U. The factor 4 was used to convert micrograms of vitamin A to International units.

The whole milk the calves received was from cows on dry feed and was analyzed monthly for vitamin A and carotene. Weekly carotene determinations were made on a composite sample of the hay that the calves received. Records were kept on feed consumption of each calf and the total carotene and vitamin A intake to 90 days of age was calculated from these data. The calves were weighed every 10 days and the number of days they scoured was recorded.

At 90 days of age all the calves were placed on a vitamin A-free ration, consisting of grain and skim milk, for the purpose of depleting their vitamin A stores. The time required for depletion was determined by following the changes in the vitamin A level of the blood plasma. A calf was considered depleted when the blood plasma contained less than 4 γ of vitamin A per 100 ml. for 2 consecutive weeks. Weekly vitamin A determinations were made on all calves from birth throughout the experimental period.

RESULTS AND DISCUSSION

The time required for the blood plasma vitamin A to decrease to 4.0 γ per 100 ml. after the various calves were placed on the depletion ration is shown in table 1. It will be noted that the two calves permitted to run with their dams on pasture required up to 4 months for depletion, whereas the calves on limited whole milk and no supplemental vitamin A usually required only 2 to 3 weeks. The exceptions in the latter group are due to the fact that some calves received hay of exceptional quality, the effect of which will be discussed later.

In most cases the calves that received supplemental vitamin A had depletion times which fell between the two extremes, depending on the amount of supplementation. Calves that received supplemental vitamin A at the rate of 50,000 I.U. per day for 50 days or more had almost the same vitamin A storage as the two calves that were reared under natural conditions. However, when either 50,000 I.U. or 25,000 I.U. of vitamin A was fed for periods shorter than 50 days, considerably less storage was observed. The depletion time of these calves generally was greater than that of the calves that received no supplement. It is not surprising, therefore, that the feeding of 5,000 to 10,000 I.U. daily in capsules had no particular effect on the health of the calves, since in these experiments it required five to ten times that quantity to materially affect the depletion time.

Considerable variation will be noted, however, (table 1) in the relation of depletion time to the amount of vitamin A fed. It should be kept in mind that the calves used were of widely different weights, as affected by individual and breed differences. They also received hay of varying carotene content and consumed

it in varying quantities. Previous data (9, 10) have shown that vitamin A and carotene requirements are proportional to body weight. Therefore, it seemed desirable to express the total vitamin A intake as a ratio with body weight and then correlate the ratio with depletion time. In the present case the ratio would be the vitamin A intake per lb. of body weight.

TABLE 1
Effect of vitamin A intake on depletion time¹

Animal no.	Treatment	Depletion time
278	With dam on pasture to 90 days of age	120
329	With dam on pasture to 90 days of age	113
708	50,000 I. U. to 98 days of age	117
513	50,000 I. U. to 92 days of age	104
2723	50,000 I. U. to 70 days of age	116
2725	50,000 I. U. to 70 days of age	149
527	50,000 I. U. to 51 days of age	94
525	50,000 I. U. to 46 days of age	59
517	50,000 I. U. to 40 days of age	98
2399	50,000 I. U. to 35 days of age	69
2713	50,000 I. U. to 30 days of age	82
712	50,000 I. U. to 30 days of age	32
713	50,000 I. U. to 30 days of age	32
515	50,000 I. U. to 30 days of age	45
2707	50,000 I. U. to 27 days of age	53
2902	25,000 I. U. to 42 days of age	40
2715	25,000 I. U. to 31 days of age	83
2718	25,000 I. U. to 31 days of age	79
2720	25,000 I. U. to 31 days of age	54
2391	25,000 I. U. to 30 days of age	74
509	25,000 I. U. to 30 days of age	66
523	25,000 I. U. to 30 days of age	67
716	25,000 I. U. to 30 days of age	30
521	No extra vitamin A	57
2714	No extra vitamin A	56
2392	No extra vitamin A	52
2597	No extra vitamin A	46
2928	No extra vitamin A	43
715	No extra vitamin A	41
512	No extra vitamin A	31
516	No extra vitamin A	30
364	No extra vitamin A	26
519	No extra vitamin A	26
518	No extra vitamin A	25
2703	No extra vitamin A	24
2594	No extra vitamin A	18
522	No extra vitamin A	18
520	No extra vitamin A	17
2700	No extra vitamin A	16
526	No extra vitamin A	15
711	No extra vitamin A	13

¹ Only 41 calves are listed, since 8 died before they were 90 days old, 2 died before they were depleted and 1 was slaughtered.

Obviously it would be advantageous to express the total carotene and the vitamin A intake in terms of International units. The carotene intake was converted to I.U. by using the figure 0.6 γ of carotene as 1 I.U. Micrograms of vitamin A were converted to I.U. by multiplying by four. The total vitamin A intake, including the carotene, is expressed in I.U. per lb. of average body weight for the first 90 days.

Using such calculations, the total vitamin A intake, including carotene, for the first 90 days was determined. From these data the average daily vitamin A intake per lb. of body weight and the depletion time were plotted in figure 1. Only 30 calves were included since data were not available on the carotene content of the milk and the hay received by 11 of the 41 calves that survived the experiment. All the various values fall fairly close to the calculated regression line, indicating a good correlation between the calculated vitamin A intake per lb. of body weight and depletion time.

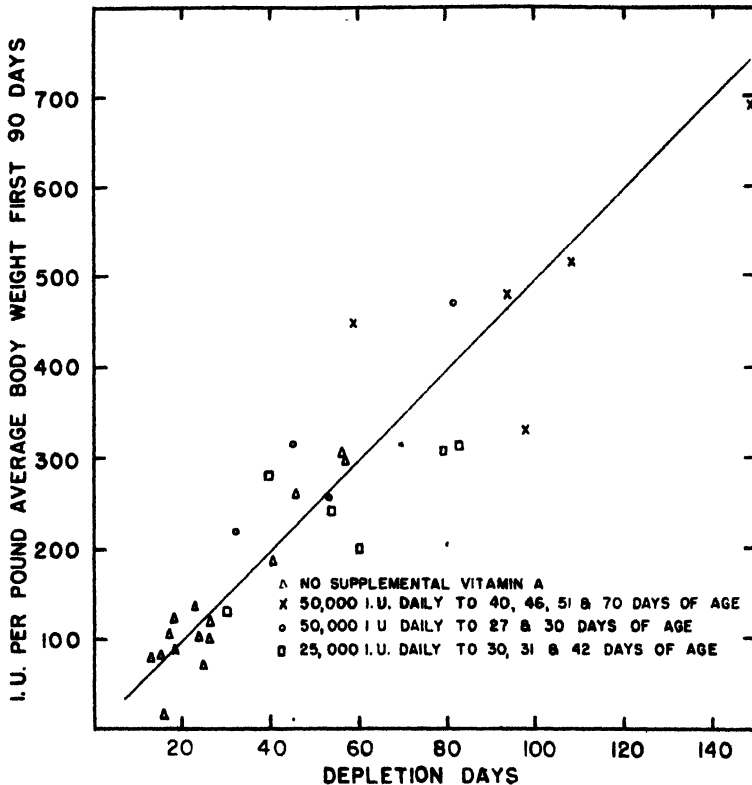


FIG. 1. The relationship between vitamin A depletion time and the total vitamin A intake per lb. average body weight first 90 days.

If it is assumed that a calf has an average weight of 100 lb. from birth to 90 days of age and that each day during this period it receives a capsule containing 5,000 I.U. of vitamin A, it would receive from the capsule 50 I.U. daily per lb. of body weight for the period. As indicated by the data in figure 1, this intake would afford only a 10-day reserve supply. If the capsules were fed for only 30 days the calf theoretically would have stored only a $3\frac{1}{2}$ day reserve from the capsules.

The relationship between carotene intake derived from hay only and deple-

tion time also has been determined for the unsupplemented calves. The average daily carotene intake per lb. of body weight has been calculated in a manner similar to that for total vitamin A intake. These data are shown in table 2. The average daily carotene intake per lb. body weight for the calves receiving no supplement was plotted against the depletion time in figure 2. The regression coefficient was found and the regression line was drawn. Larger carotene intake from the hay was associated with greater storage of vitamin A and longer depletion time.

Previously published data (9, 10) showed that a carotene intake of 30 γ for Holsteins and 34 γ for Jerseys per lb. of body weight were the minimums necessary to maintain a normal spinal fluid pressure in growing calves 4 to 14

TABLE 2

Relation of the carotene intake from hay, per pound of body weight to the depletion time of the non-supplemented calves¹

Calf no.	Av. wt. first 90 d.	Hay consumed first 90 d.	Av. carotene content of hay	Total carotene intake from hay	Av. daily carotene intake/lb. body wt.	Depletion time
	(lb.)	(lb.)	(γ /g.)	(mg.)	(γ)	(d.)
2714	69.0	53.3	42.6	1031.3	166.1	56
521	93.5	70.9	42.6	1371.6	163.0	57
2597	147.5	89.1	48.9	1979.6	149.1	46
715	164.5	117.0	26.7	1419.5	95.9	41
364	140.0	65.2	33.8	1000.4	79.4	26
516	102.5	73.1	18.4	611.4	66.3	30
522	85.0	25.0	44.4	503.5	65.8	18
519	98.0	63.4	18.2	526.1	59.7	26
2703	97.0	53.8	15.9	387.3	44.4	24
711	121.0	45.7	18.6	386.3	35.5	13
520	80.0	44.2	13.8	277.2	38.5	17
2594	142.5	69.8	16.3	545.7	38.2	18
526	80.5	24.0	22.3	242.7	33.5	15
518	68.5	14.1	18.1	121.1	19.8	25
2700	66.5	9.3	22.3	94.4	15.8	16

¹ Three of the non-supplemented calves are not shown as the carotene content of the hay they consumed was not available.

months of age. The four calves which received carotene at about the minimum, as determined in older calves, and received in addition the vitamin A and carotene in a limited feeding of whole milk, showed only about a 2-week supply (table 2) at 90 days of age. Even doubling this intake (calves 364, 516, 522) only increased the depletion time approximately 7 days. Probably minimum requirements should be increased several-fold for optimum results. This point is emphasized further by the fact that the two calves that suckled their dams while on pasture had sufficient storage at 90 days of age to carry them for eight to nine times longer.

From figure 2 and table 2, it also is obvious that the quality and quantity of the hay fed are very important items in rearing a calf so that its body stores of vitamin A will be more nearly normal. The calves that received the hay with the higher carotene content stored more vitamin A and, therefore, had a

greater depletion time. It also should be pointed out that the hay fed to the calves in this experiment had a higher carotene content than average hay. Moreover, the calf feeder was diligent in picking out the best bales of hay from the best lots of purchased hay.

Under farm conditions, most hay would average about 15 γ or less of carotene per g. of hay. Only one calf in this experiment received hay with an aver-

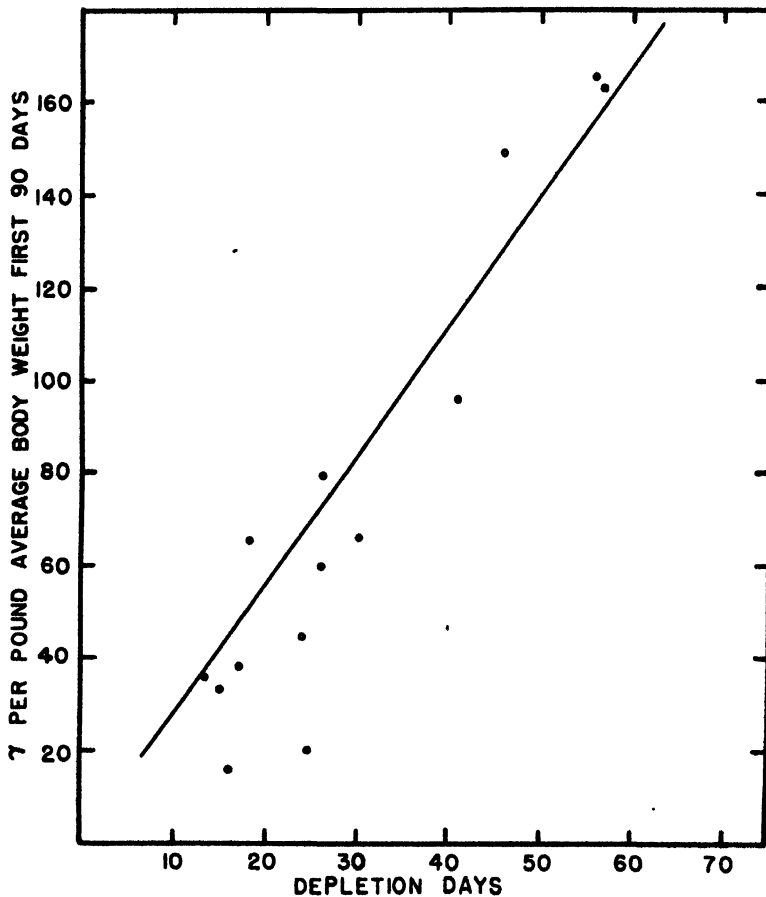


FIG. 2. The relationship between vitamin A depletion time and the carotene intake per lb. average body weight the first 90 days.

age carotene content below this figure, as shown in table 2. One might wonder how much vitamin A the calves would have stored if they had received hay that averages only 10 γ per g., which still would be a good quality of hay. However, in this experiment even the calves that got the very best hay did not have nearly so great a store of vitamin A as those reared under natural conditions with the dams.

Table 3 shows the average level of vitamin A in the blood plasma of the

various groups of calves at various ages. The plasma values for the non-supplemented group are about half those of some of the other groups up to 80 days of age. The increase in these calves and in those which received 25,000 I.U. of vitamin A as a supplement, which is evident at about 80 days of age, is probably a reflection of increased hay intake at this time. The group that received supplemental vitamin A at the rate of 50,000 I.U. per day for the first 50 to 98 days had vitamin A blood levels that corresponded to blood levels encountered in calves reared under natural conditions. The groups of calves which were supplemented for only 30 days had low blood vitamin A values up to 60 or 70 days of age, even when they had been supplemented at the rate of 50,000 I.U. per day. Apparently to maintain a level of vitamin A in the blood plasma of dairy calves reared under present day feeding practices equal to those reared under natural conditions, about 50,000 I.U. of vitamin A must be fed each day until the calf reaches the age of 50 or 70 days.

The average weight gain is summarized in table 4. In the Holstein group, there is little difference in weight gain between the non-supplemented calves

TABLE 3
Effect of supplemental vitamin A on average vitamin A level in the blood plasma

Age	No extra vitamin A	25,000 I. U. 27 & 30 days	50,000 I. U. 27, 30, 40 & 46 days	50,000 I. U. 51, 70, 92 & 98 days	Pasture with dam
(d.)	18 calves	8 calves	8 calves (γ /100 ml.)	5 calves	2 calves
8-21	7.5 \pm .4	9.4 \pm .5	10.0 \pm .5	12.4 \pm .7	12.9
22-35	5.8 \pm .3	8.3 \pm .3	9.6 \pm .7	12.4 \pm .9	11.5
36-49	5.7 \pm .3	8.2 \pm .5	9.8 \pm .9	13.9 \pm .8	10.4
50-63	5.8 \pm .4	7.2 \pm .7	9.3 \pm .6	13.1 \pm .8	11.8
64-77	7.6 \pm .5	9.3 \pm .5	10.0 \pm 1.0	12.7 \pm .7	11.2
78-91	10.1 \pm .7	12.0 \pm .5	9.8 \pm 1.0	13.3 \pm .8	12.5
92-105	9.5 \pm .7	15.2 \pm 1.1	11.3 \pm .6	14.4 \pm .7	12.1

and the supplemented calves, but the number of Holstein calves is too small for a good comparison. The Jerseys, on the other hand, show some differences in weight gain. The Jerseys which received the supplemental vitamin A gained on the average 9 lb. more than the non-supplemented group. Statistical treatment of this difference showed no significant difference.

The Jersey calves that received no supplemental vitamin A during the first 90 days scoured on an average of 12 days per calf, while the supplemented Jersey calves scoured on an average of 9 days per calf. The Holstein calves that received no supplement scoured on an average of 7 days per calf, while the supplemented Holstein calves scoured on an average of 2.5 days per calf.

Mortality may be a better measure of the value of supplemental vitamin A than data on the frequency of scours, since it is rather difficult to measure the severity of the scours. In this experiment, 2 calves out of 23 in the group that received supplemental vitamin A died of pneumonia and scours before they reached 90 days of age, while 6 out of 24 calves in the non-supplemented group died before they reached 90 days of age.

In general the data presented in this paper indicate that the feeding of fairly large dosages of vitamin A may be of some value. However, the results should be checked further before a general overall recommendation is made to dairymen to feed large dosages of vitamin A. As a matter of fact, the use of sulfa drugs to control diarrhea and pneumonia may be a more practical solution of the problem. Sanitation is also a most important factor in the control of scours and pneumonia in calves. It has been amply demonstrated that mortality can be reduced materially by proper sanitation and management. It seems possible that where sulfa drugs and proper sanitation and management practices are used the amounts of vitamin A needed to rear calves successfully may be less than when sulfa drugs are not used or where poor sanitation is prevalent.

It may be argued that a large number of calves is reared each year on a limited whole milk-feeding program without any supplemental vitamin A. Yet it is known that in many large herds mortality can run high and it may be that the bacteria causing scours or pneumonia have an opportunity to build up virulence by passing from calf to calf. One might ask if the vitamin A intake

TABLE 4
Comparison of total gain to 1, 2 and 3 months of age of the supplemented and non-supplemented calves

Breed	Treatment	No. of calves	Av. body wt.	Av. total gain 1st mo.	Av. total gain to 2 mo.	Av. total gain to 3 mo.
			(lb.)	(lb.)	(lb.)	(lb.)
Jersey	No supplemental vit. A	14	57	15 ± 1.5	31 ± 2.9	50 ± 4.4
Jersey	Supplemental vit. A	16	58	15 ± 1.7	38 ± 2.5	59 ± 5.1
Holstein	No supplemental vit. A	6	93	20 ± 2.4	55 ± 5.8	95 ± 8.6
Holstein	Supplemental vit. A	5	96	16 ± 1.7	57 ± 4.1	95 ± 24.2

had been high in the first place, would the bacteria have had the chance to build up virulence? While the literature on the relationship of vitamins to resistance to disease is not very encouraging, a few questions in this direction need to be answered with calves. However, information is needed on the bacteria or viruses that may cause scours and pneumonia in calves, in order to be able to conduct properly-controlled experiments and evaluate correctly the value of supplementation.

Whether or not the amounts of vitamin A, which have been shown in the present paper to be necessary to maintain what may be considered to be maximum stores and blood levels of vitamin A, are necessary to raise calves successfully under present-day conditions of feeding cannot be determined with the data available. The data do suggest that feeding 5,000 to 10,000 I.U. of vitamin A for relatively short periods of time can have little effect on the amounts of vitamin A stores even though these amounts would prevent the appearance of gross deficiency symptoms. Therefore, it is not surprising that several groups of investigators (4, 11) have been unable to demonstrate any beneficial effects on the incidence of scours by feeding capsules containing 5,000 or 10,000 I.U. of vitamin A.

The proper technic to use in determining the relative quantity of storage of vitamin A in the calf is open to question. It has been observed in using other technics, as well as in the present technic, that the level of blood plasma vitamin A in itself is not a good indication of storage where greater-than-minimum requirements of vitamin A or carotene are fed. In most previous experiments the calves have been slaughtered and the livers analyzed for vitamin A. The disadvantage of this technic is that the calf is not available for further use. Also it might be questioned whether calves with the same or widely different body stores of Vitamin A would utilize the reserves with the same efficiency.

Previously, Moore and Berry (8) had suggested the technic used in this study. In the present study, as the vitamin A of the plasma approached the level of 4.0 γ per 100 ml., the calves usually showed a decreased rate of gain. This decrease in rate of gain is additional evidence that the vitamin A stores are about depleted. The technic appears valid since there is a good correlation between intake and depletion time. The correlation was especially good in the non-supplemented group as shown by the grouping of the data in figure 2.

In presenting the data in figures 1 and 2, in which the calculated intake of vitamin A and carotene have been plotted against depletion time as a straight line, it is realized that exceptionally large dosages would not be utilized as efficiently as smaller ones and under certain conditions of supplementation this straight line relationship would not hold. Apparently the dosages used in these experiments were utilized with about equal efficiency.

SUMMARY

1. Calves that received varying quantities of vitamin A for the first 90 days of age then were given a vitamin A-deficient ration to determine the time required to deplete their stores. The stores were considered depleted when the blood plasma vitamin A values reached 4.0 γ per 100 ml.
2. Two calves that were permitted to run with their dams on pasture required 4 months on the ration deficient in vitamin A to deplete their stores.
3. Calves reared according to present methods of limited whole-milk feeding with hay of above average quality required a depletion time of 2 to 4 weeks.
4. The feeding of hay of exceptionally good quality (high in carotene content) increased the depletion time up to 6 to 8 weeks.
5. Fifty thousand I.U. of vitamin A for 50 or more days, in addition to the vitamin A received in the feed, was necessary in order to maintain stores and blood levels equal to those of calves reared with the dams on pasture. The depletion time of these calves was from 3.5 to 4 months. Feeding 25,000 or 50,000 I.U. of vitamin A under similar conditions for periods of 30 to 45 days reduced the depletion time to intervals ranging from 30 to 98 days.
6. The groups that received supplemental vitamin A showed somewhat better gains, fewer cases of scours, and less mortality than the non-supplemented groups.
7. The technic of determining the number of days after 90 days of age that were required for the blood plasma values to decrease to 4.0 γ per 100 ml. appears to give a good estimation of the vitamin A stores.

REFERENCES

- (1) CONVERSE, H. T., AND MEIGS, E. B. Some disasters in reproduction and growth caused by low quality hay. *Proc. Am. Soc. Animal Production*. 1932.
- (2) CONVERSE, H. T., AND MEIGS, E. B. The vitamin requirements for normal growth in young dairy cattle. *Proc. Am. Soc. Animal Production*, 32nd Ann. Rpt., pp. 67-72. 1939.
- (3) GULLICKSON, T. W., AND FITCH, J. B. Effect of adding cod-liver oil to the rations of dairy calves. *J. Dairy Sci.*, 27: 331-335. 1944.
- (4) KRAUSS, W. E., HIBBS, J. W., AND SUTTON, T. S. Supplementary vitamins for calves. *Feed Stuffs*, 17: 40-43. 1945.
- (5) KRAUSS, W. E., MONROE, C. F., AND HAYDEN, C. C. The value of milk fat substitute-skim-milk combination for raising bull calves for veal and heifer calves for replacement. *Ohio Agr. Expt. Sta. Spec. Cir.* 56. 1939.
- (6) LEWIS, J. M., AND WILSON, L. T. Vitamin A requirements of calves. *J. Nutrition*, 30: 467-477. 1945.
- (7) MOORE, L. A. Relationship between carotene, blindness due to constriction of the optic nerve, papillary edema and nyctalopia in calves. *J. Nutrition*, 17: 443-459. 1939.
- (8) MOORE, L. A., AND BERRY, M. H. Vitamin A and carotene content of the blood plasma of dairy calves from birth up to four months of age. *J. Dairy Sci.*, 28: 821-826. 1945.
- (9) MOORE, L. A., BERRY, M. H., AND SYKES, J. F. Carotene requirements for the maintenance of a normal spinal fluid pressure in dairy calves. *J. Nutrition*, 26: 649-658. 1943.
- (10) MOORE, L. A., SYKES, J. F., JACOBSON, W. C., AND WISEMAN, H. G. Carotene requirements for Guernsey and Jersey calves as determined by spinal fluid pressure. *J. Dairy Sci.*, 31: 533-538. 1948.
- (11) NORTON, C. L., EATON, H. D., LOOSLI, J. K., AND SPIELMAN, A. A. Controlled experiments on the value of supplementary vitamins for young dairy calves. *J. Dairy Sci.*, 29: 231-238. 1946.
- (12) PHILLIPS, P. H., LUNDQUEST, N. S., AND BOYES, P. D. The effect of vitamin A and certain members of the B complex upon calf scours. *J. Dairy Sci.*, 24: 977-982. 1941.
- (13) WISE, G. H., CALDWELL, M. J., PARRISH, D. B., ATKINSON, F. W., AND HUGHES, J. S. Relation of typical postnatal changes in the diet of the dairy calf to the concentration of carotenoids and vitamin A in the blood serum. *J. Animal Sci.*, 7: 70-77. 1948.

FILLED MILKS FOR DAIRY CALVES. I. SOYBEAN OIL VERSUS MILK FAT¹

N. I. JACOBSON, C. Y. CANNON AND B. H. THOMAS

Iowa State College

One of the major expenses in raising dairy calves is the whole milk fed. Fairly good growth can be obtained when calves are fed skim milk supplemented with hay and grain (1, 4), although young calves fed in this manner do not gain weight so rapidly as those fed whole milk and do not exhibit so thrifty an appearance (4). During the first month after birth, when the calf eats very little hay and grain, milk fat is important, especially as a source of energy, since it has about one-half the total digestible nutrient value of whole milk. Thus the presence of fat in the diet of the young calf is desirable for optimum development.

The question then arises as to whether a milk in which a less expensive fat or oil has been substituted for the butterfat might be equally as satisfactory as whole milk as a feed for young calves. Skim milk into which tallow or lard has been homogenized has proved satisfactory as a feed for young calves (3, 4). The growth and general appearance of animals fed such a milk, however, have been inferior to those of calves fed whole milk. Vegetable fats, which generally are less expensive than animal fats, have proved unsatisfactory in calf feeding (1, 3, 4). One of the oils that has been least satisfactory and which recently has become an important agricultural by-product in the Midwest is soybean oil. Previous experimental work has shown that feeding filled milk containing 3 per cent or more soybean oil to young dairy calves produces poor growth and excessive scouring (1, 3, 4, 5). The diarrhea commonly was in evidence within a few days after the introduction of soybean oil into the ration. Weakness, emaciation and high mortality result when calves are fed such a soybean oil-filled milk for an extended period of time.

In preliminary work at this station (5), an attempt was made to improve the nutritional value of soybean oil-filled milk by various modifications of the product prior to feeding. These modifications were (a) partial hydrolysis of the soybean oil immediately prior to feeding by the addition of lipase, (b) addition of a buffer solution, (c) inclusion of additional carbohydrate in the form of dextrinized starch, (d) addition of finely-ground alfalfa leaf meal, (e) addition of emulsifying agents and (f) addition of rumen fluid from a cow that was fed a ration composed of alfalfa hay and a concentrate mixture. These modified soybean oil-filled milks enhanced neither growth nor general appearance of young dairy calves to which they were fed.

Gullickson (2) and Gullickson and Fitch (3) suggested that a possible explanation for the poor growth of calves fed filled milks containing crude

Received for publication December 21, 1948.

¹ Journal Paper No. J1618 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 814.

vegetable oils may be that these oils interfere either with utilization of B-complex vitamins by the calf or with the bacterial synthesis of these nutrients in the digestive tract. These authors also indicate that calves fed a filled milk containing "Crisco", a hydrogenated cottonseed oil, gained weight more rapidly than calves that received a filled milk containing the unhydrogenated product.

In view of the results of Gullickson and Fitch (3) and the preliminary work at this station (5), the experiment reported herein was designed to investigate this problem further.

EXPERIMENTAL

Thirty-five calves from the Iowa State College dairy herd were allotted to seven groups. Four of the calves in each group were Holsteins or Holsteins and Brown Swiss, and the fifth was an Ayrshire, Guernsey, Jersey or Milking Short-

TABLE 1
Ingredients of rations fed experimental calves

Group	Rations ^a					
	Whole milk ^b	Dried skim milk	Butter oil	Crude expeller soybean oil ^c	Hydrogenated soybean oil ^d	Water
	(%)	(%)	(%)	(%)	(%)	(%)
I	100					
II		10	3			87
III		10				90
IV		10		2		88
V		10		3		87
VI ^e		10		3		87
VII		10			3	87

^a All groups received daily supplements of vitamins A and D and a mineral mixture.

^b Approximately 3 per cent fat.

^c Produced by Swift and Company Soybean Mill, Des Moines, Iowa. Melting point approximately 40° F.; iodine number, 130 (7).

^d Produced by Swift and Company, Chicago, Illinois. Melting point 100–103° F.; iodine number, 75 (7).

^e Supplemented with 10 mg. thiamine, 10 mg. riboflavin, 10 mg. pyridoxine, 50 mg. calcium pantothenate, 100 mg. nicotinic acid amide, 250 mg. ascorbic acid and 50 mg. mixed tocopherols per calf daily.

horn. Within these two breed groupings, the assignment of calves to experimental groups was at random.

Each calf was allowed to remain with its dam for 3 days immediately following birth. On the fourth day, the calf was transferred to the experimental milk, which was given at the rate of 10 lb. per 100 lb. body weight per day. The experimental milks were fed twice daily from nipple-type pails. When calves began to scour, there was no reduction or modification of the ration. The amount of milk fed to each calf was adjusted each time the calf was weighed. The weight and the height at withers of each calf were determined at the age of 4 days and weekly thereafter. The calves, placed in individual pens, were muzzled at all times except when being fed. The calves were given no feeds other than experimental milks and vitamin and mineral supplements for the duration of the experiment.

Spielman *et al.* (9) successfully controlled infectious scours in young calves by the daily oral administration of 4 to 8 g. of "sulfathalidine" per calf during the first 7 days after birth. In this experiment each calf received 6 g. of "sulfathalidine" per 100 lb. body weight daily, administered in two equal doses prior to each feeding for the first 10 days following birth.

All milks (table 1) except whole milk were homogenized at approximately 3,000 lb. pressure. The whole milk was obtained twice daily from Holstein cows producing milk averaging 3 per cent fat. All milks were fed at a temperature of approximately 37° C.

Each calf received daily a gelatin capsule containing approximately 5,000 U. S. P. units of vitamin A and 1,000 U. S. P. units of vitamin D. In addition, approximately 25,000 U. S. P. units of vitamin A per 100 lb. body weight were fed to all calves daily during the first 10 days of the experimental period. In the latter case, the fish liver-oil concentrate containing the vitamin A was added to the milk prior to homogenization. In the case of the whole milk, which was unhomogenized, the vitamin A supplement was mixed with the milk by vigorous hand-stirring. All calves received a mineral mixture² in capsules at the rate of 7 g. daily per 100 lb. body weight.

Observations for evidence of scouring were made each time the calves were fed.

An analysis of variance and the t-test (8) were employed in the statistical evaluation of the data.

RESULTS AND DISCUSSION

The data regarding the growth and general health of calves fed the various rations are presented in table 2. Gains in weight and increases in height at withers are expressed as per cent of initial values.

The recommended daily allowance of total digestible nutrients for a 100-lb. calf is 2 lb. (6). It generally is recommended that calves be fed no more than 10 lb. of milk daily per 100 lb. body weight, since higher levels sometimes cause indigestion and scouring. Therefore, all calves in this experiment were fed at the latter level, even though the total digestible nutrients supplied were less than the recommended intake. Normally, at 2 to 4 weeks of age, calves begin eating hay and grain. In the experiment reported herein, hay and grain were excluded from the rations because of the variability among individual animals in the rate of consumption of these feeds. All calves, except a few individuals that developed severe indigestion and scouring, drank the prescribed quantity of milk. The filled milk containing 3 per cent fat provided, according to calculations, 1.5 lb. of total digestible nutrients daily per 100 lb. body weight. The milk containing 2 per cent oil and the reconstituted skim milk supplied 1.3 and 0.8 lb., respectively, of total digestible nutrients.

There were no statistically significant differences in mean gain in weight, in mean increase in height at withers or in incidence of scouring among the groups

² The mineral mixture was composed of the following: Tricalcium phosphate, 400 parts; sodium chloride, 200 parts; magnesium oxide, 15 parts; calcium carbonate, 11 parts; potassium chloride, 11 parts; ferrous sulfate, 2 parts; manganous sulfate, 0.5 part; zinc oxide, 0.5 part; potassium iodide, 0.5 part; cobaltous acetate, 0.5 part; copper sulfate, 0.2 part.

TABLE 2

Growth and incidence of scouring of calves fed whole or various reconstituted milks during an eight-week experimental period

Dietary Groups	Breed ^a	Initial wt. (4 d.)	% gain in wt.	Initial height (4 d.)	% gain in height	Incidence of scouring ^b	Condition ^c
I		(lb.)		(cm.)		(%)	
Butterfat, 3% (whole milk)	H	105	41	75.0	12.7	0.0	Excellent
	H	100	35	77.0	9.7	3.5	Excellent
	H	88	32	72.5	7.6	3.5	Excellent
	G	96	17	75.0	9.3	8.0	Very good
	H	63	32	68.0	11.8	0.0	Very good
	Av.	90.4	31.4	73.5	10.2	3.0	
II							
Butter oil, 3%	H	81	38	73.5	8.8	0.0	Excellent
	H	92	48	75.5	7.9	3.5	Excellent
	A	103	32	76.5	10.5	0.0	Excellent
	H	92	38	75.0	7.3	9.7	Excellent
	H	92	45	77.0	9.1	0.9	Excellent
	Av.	92.0	40.2	75.5	8.7	2.8	
III							
Low fat (reconstituted skim milk)	H	90	18	75.5	9.3	0.0	Very good
	G	87	-16	75.5	0.7	4.4	Fair
	H	106	12	77.0	5.2	1.8	Very good
	H	88	-5	75.0	4.0	8.0	Fair
	H	95	7	77.0	3.9	3.5	Good
	Av.	93.2	3.2	76.0	4.6	3.5	
IV							
Crude expeller soybean oil, 2%	H	81	20	71.5	9.1	6.2	Good
	BS	85	8	74.5	4.0	15.0	Fair
	J	47	-8	64.5	5.4	38.1	Poor
	H	100	23	75.0	6.7	10.6	Very good
	H	93	19	76.5	4.6	25.7	Very good
	Av.	81.2	12.4	72.4	6.0	19.1	
V							
Crude expeller soybean oil, 3%	H	100	-19	75.0	0.0	46.7	Died (13 d.)
	BS	92	22	74.0	8.8	23.9	Fair
	MS	87	-10	74.0	2.0	58.8	Died (11 d.)
	H	76	-11	73.0	-1.4	65.3	Died (40 d.)
	H	83	29	71.5	8.4	24.8	Fair
	Av.	87.6	2.2	73.5	3.6	43.9	
VI							
Crude expeller soybean oil, 3% + vitamin supplement	H	104	35	77.5	7.7	21.2	Good
	J	47	-13	63.0	-1.6	45.5	Poor ^d
	H	96	21	77.0	8.4	25.7	Fair
	H	54	-22	67.0	0.0	50.0	Died (12 d.)
	BS	92	-9	74.5	3.4	48.4	Died (52 d.)
	Av.	78.6	2.4	71.8	3.6	38.2	
VII							
Hydrogenated soybean oil, 3%	BS	111	35	78.0	10.3	2.7	Excellent
	A	71	35	70.0	10.0	11.5	Excellent
	H	80	51	73.5	11.6	0.9	Excellent
	H	78	47	72.0	10.4	4.4	Excellent
	H	80	41	71.0	8.5	0.9	Excellent
	Av.	84.0	41.8	72.9	10.2	4.1	

^a A, Ayrshire; BS, Brown Swiss; G, Guernsey; H, Holstein; J, Jersey; MS, Milking Shorthorn.

^b Observations made for scouring at each feeding.

Incidence of scouring = $\frac{\text{Number of times scouring observed}}{\text{Total number of examinations}} \times 100.$

^c Estimate of physical condition at end of expt.

^d Removed from expt. at 9d. to save life.

fed whole milk (group I), reconstituted milk containing butter oil (group II) and a filled milk containing hydrogenated soybean oil (group VII). All calves receiving the foregoing rations were in good physical condition at the end of the experimental period.

A comparison of the mean weight gains of calves receiving whole milk with the mean weight gains of those fed reconstituted skim milk and of those fed the various rations containing crude expeller soybean oil showed the differences to be significant statistically at the 1 per cent and the 5 per cent levels of probability, respectively. When the mean weight gains of the calves fed reconstituted milk containing butter oil (group II) and those fed a filled milk containing hydrogenated soybean oil (group VII) were compared to those of calves fed the other rations (excluding whole milk), the differences in all cases were significant at the 1 per cent level of probability.

The mean per cent increase in height at withers among the groups receiving whole milk (group I), reconstituted milk containing butter oil (group II) and filled milk containing hydrogenated soybean oil (group VII) differed significantly from those of groups receiving reconstituted skim milk (group III) and filled milk containing 2 per cent expeller soybean oil (group IV).

The reconstituted skim milk ration (group III) was included in the experiment to determine whether the fats incorporated in the various rations improved the growth of the calves. In general, the calves fed skim milk maintained weight and exhibited relatively little skeletal development as measured by height at withers. This retarded growth probably was due to the smaller total digestible nutrient intake of these calves.

Calves that were fed a filled milk containing 3 per cent crude expeller soybean oil (group V) exhibited poor growth and excessive scouring. Three of the calves in this group died before the end of the 8-week experimental period. Gullickson and Fitch (3) stated that calves fed vegetable oils "showed a marked benefit" from the supplementation of the ration with various members of the vitamin B-complex, but these authors did not specify the vitamins fed. One group of calves (group VI) in the present investigation received a vitamin supplement containing thiamine, riboflavin, nicotinic acid, calcium pantothenate, pyridoxine, ascorbic acid and mixed tocopherols. No improvement in growth or physical condition of the calves resulted from the supplementation of filled milk containing 3 per cent crude expeller soybean oil with liberal amounts of these vitamins. Two of the calves in this group died during the experiment and a third probably would have died had it not been removed from the experiment.

Post-mortem examinations were made of the five calves which died during the experiment.³ Pneumonia was observed in two calves (those which died at 40 days and at 52 days of age, respectively), but in the other three, pathogenic organisms apparently were absent. The symptoms most frequently exhibited prior to death were severe scouring, emaciation, anorexia, weakness, abdominal pain, stiffness of the rear quarters and severe dehydration.

³ Post-mortem examinations conducted by Iowa Veterinary Diagnostic Laboratory, Iowa State College, Ames, Iowa.

In general, calves fed a filled milk containing crude expeller soybean oil at a level of 2 per cent (group IV) grew better, as judged by increases in body weight and in height at withers, and scoured less frequently than those fed the rations containing 3 per cent crude expeller oil. This suggests a possible tolerance level for the oil. However, the marked within-group variations indicate that some calves were more susceptible than others to the adverse effects resulting from the ingestion of crude expeller soybean oil.

The results of this investigation do not explain why unsatisfactory growth occurs when young calves are fed filled milks. However, the fact that hydrogenated soybean oil can be fed successfully, suggests that the first step in the solution of this problem should be the clarification of the cause for the differences in growth response of young calves fed hydrogenated soybean oil-filled milk as compared with those fed crude expeller soybean oil-filled milk. The results of experiments designed to clarify this problem further will be reported later.

SUMMARY

Under the conditions of this experiment, a filled milk containing hydrogenated soybean oil produced growth in young dairy calves equal to the growth of calves fed whole milk. There was no significant difference in gain in weight, in increase in height at withers, in incidence of scouring or in physical appearance between the two groups. The growth and general appearance of the group of calves fed hydrogenated soybean oil were in sharp contrast to those of the group fed crude expeller soybean oil, the latter being characterized by poor growth, severe scouring and high mortality. Supplementation of the rations of young dairy calves fed a filled milk containing 3 per cent crude expeller soybean oil with various members of the vitamin B complex, ascorbic acid and mixed tocopherols failed to improve growth or reduce the incidence of scouring.

REFERENCES

- (1) ESPE, D. L. Iowa Agr. Expt. Sta. Unpublished. 1946.
- (2) GULLICKSON, T. W. Minn. Agr. Expt. Sta. Private communication. 1946.
- (3) GULLICKSON, T. W., AND FITCH, J. B. Tests Show Young Calves Need Butterfat. Minn. Farm & Home Sci., 3: 12-13. 1946.
- (4) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves. J. Dairy Sci., 25: 117-128. 1942.
- (5) JACOBSON, N. L., ESPE, D. L., CANNON, C. Y., AND THOMAS, B. H. Iowa Agr. Expt. Sta. Unpublished. 1946.
- (6) LOOSLI, J. K., HUFFMAN, C. E., PETERSEN, W. E., AND PHILLIPS, P. H. Recommended Nutrient Allowances for Domestic Animals. No. III Recommended Nutrient Allowances for Dairy Cattle. National Research Council, Washington, D. C. 1945.
- (7) MITCHELL, H. S. Swift and Company, Chicago, Ill. Private communication. 1947.
- (8) SNEDECOR, G. W. Statistical Methods. 4th Ed. Iowa State College Press, Ames, Iowa. Ch. 3, 4 and 10. 1946.
- (9) SPIELMAN, A. A., LOOSLI, J. K., THOMAS, J. W., NORTON, C. L., AND TURK, K. L. Carotene Utilization in the Newborn Calf. J. Dairy Sci., 29: 381-391. 1946.

MILK LIPASE SYSTEM. II. COMPARISON OF SOLVENT EXTRACTION AND CHURNING METHODS FOR OBTAINING FAT FROM MILK FOR FREE FATTY ACID MEASUREMENT.¹

B. C. JOHNSON AND I. A. GOULD²

Maryland Agricultural Experiment Station, College Park

The direct titration of milk fat to measure the free fatty acid content is a widely-used procedure for determining the extent of lipolysis in milk and its products. It was established by Gould and Trout (4) that this procedure is a far more sensitive means of detecting lipolysis than by titration of the milk or by pH measurements. Later, Herrington and Krukovsky (5) reported that when titration was conducted on the fat itself rather than upon the milk, it was not necessary to correct for non-fatty acidity due to proteins, salts and moderate amounts of lactic acid.

Although many workers have utilized fat titers (acid degrees) as a basis for measuring lipase activity, little consideration has been given to methods of removing the fat from milk for analysis. The general practice has been to churn the milk or cream, then purify the fat by melting, centrifuging and filtering. The weakness of this method of obtaining the fat lies in the loss of the water-soluble and flavor-producing free fatty acids in the buttermilk. This loss was indicated by Gould (2), who found that fat obtained by churning rancid milk did not possess a rancid flavor even though the free fatty acid content was extremely high.

Efforts to associate changes in fat characteristics with lipolysis have been, in general, unsuccessful (4, 7). However, in such studies the fat for analysis was obtained by churning. Even a six- to nine-fold concentration of the free fatty acids by alcohol extraction of the fat did not permit the differentiation between rancid and non-rancid fat on the basis of Reichert-Meissl, Polenske, saponification and iodine values (4). Possibly such measurements may be revealing if the fat is obtained from the milk in a manner to retain the lower fatty acids which have been freed by lipase action.

In earlier unpublished studies the observation was made that extraction of milk or cream with solvents may yield more complete recovery of the fatty acids than is obtained by churning. The development and application of such a method constitutes the basis for this paper. An earlier abstract dealt with a portion of these findings (8).

PROCEDURE

Fresh, raw milk from the University herd was used in all studies. Lipolysis was accelerated by homogenizing with a rotary homogenizer and storing for

Received for publication January 10, 1949.

¹ Scientific paper no. A224. Contribution no. 2149 of the Maryland Agricultural Experiment Station (Dairy Department).

² Present address: Dairy Technology Dept., Ohio State University, Columbus.

several hours at 35 to 40° F. (3). The rancid milk was separated to give about 30 per cent cream and the cream heated to 65° F. for 10 minutes to stop further lipase action. Control or non-rancid cream was produced in the same manner, but with homogenization omitted.

Methods of obtaining fat. Purified milk fat for chemical analysis was obtained from the cream either by the churning method or by solvent extraction. In the former method, the cream was churned, the butter granules washed free of buttermilk with a minimum of cold water, the butter melted and centrifuged and the clear fat layer removed by siphonation and purified by filtration through paper.

The solvent extraction procedure was as follows: To 125 g. of cream in a 1-l. Erlenmeyer flask were added 100 ml. of ethanol. The flask was stoppered and shaken vigorously for 15 seconds and allowed to stand for 5 minutes to aid in extraction. A total of 200 ml. of the extractants then was added and the mixture shaken vigorously for 30 seconds. The extractants were ethyl ether and Skellysolve F.³ Trials were conducted to determine the proper proportion of the two extractants and whether they should be added together or separately. Following the addition of the solvents, centrifugation was used to break the emulsion and the ethereal layer was removed by siphonation. In the earlier portion of the study, the solvents were vaporized on a hot plate at 135° C. until bubbles ceased to rise and the last traces were removed by heating the fat at 100° C. under reduced pressure of about 100 mm. mercury for 10 minutes. It later was demonstrated that lower temperature and higher vacuum permitted more efficient recovery of the lower fatty acids.

Analysis of fat. Chemical analyses were conducted on the fat obtained by churning and by extraction. The methods used were those of the A.O.A.C. (1), except for such changes as noted. Reichert-Meissl, Polenske, iodine (Hanus) and saponification values and acid degree were determined.

The apparatus used for the Reichert-Meissl and Polenske determinations was constructed with ground glass connections, and six analyses (four fat samples and two blanks) were conducted simultaneously. Small pieces of porous plate, about 2 mm. in diameter, and one or two small glass beads added before saponification were found to be more effective than pumice as boiling stones.

Additional chemical determinations were made upon the various fractions resulting from extraction of the fat with boiling ethanol. The method used for ethanol extraction of milk fat is as described by Gould (3) with the exception that the fat was obtained by solvent extraction rather than by churning.

EXPERIMENTAL RESULTS

Development of the solvent extraction procedure. To find the ratio of solvents which would yield the most efficient extraction of the fat, the proportions of ethyl ether to Skellysolve were varied in the ratios of 4:0, 3:1, 1:1, 1:3 and 0:4. The method of addition and mixing the solvents with the cream-alcohol mixture was varied, the solvents being added either separately with 15-second

³ A petroleum solvent, boiling range 30–60° C.

shaking after each addition or together followed by 30-second shaking. The results obtained in this portion of the study are shown in figure 1.

In general, the acid degree of the fat increased and the recovery of the fat decreased as the amount of Skellysolve increased. The most uniform results occurred when the ratio of solvents was in the range of 3:1 to 1:3 ethyl ether to Skellysolve. In general, when the ratio of ether to Skellysolve was above 1:1 and often at 1:1, there was retention of water and solids-not-fat in the ethereal extract. This condition was observed consistently when the proportion of 3:1 (ether to Skellysolve) was used and was pronounced when ether was used alone. On the

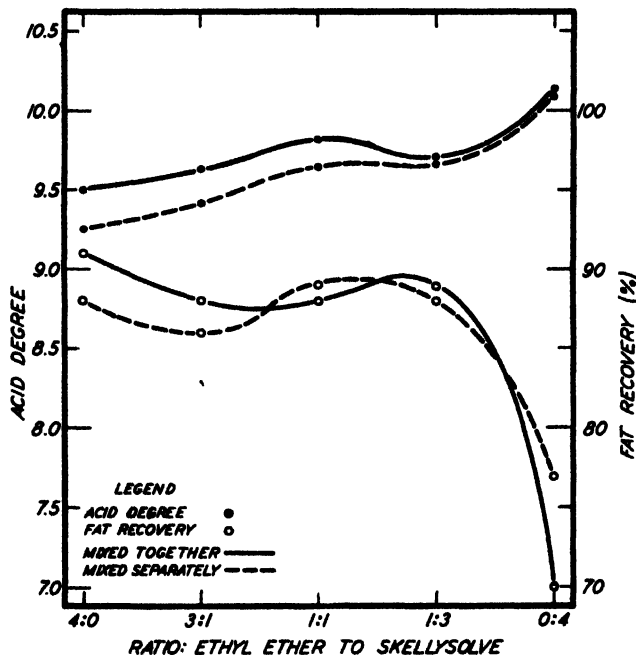


FIG. 1. Effect of varying the method of mixing and the ratio of solvents upon the acid degree and percentage recovery of milk fat by the solvent extraction method.

other hand, when Skellysolve alone was used as the extractant, low recovery of fat nearly always was encountered, accompanied by high and inconsistent acid values of the fat.

The data also indicate that slightly higher and more uniform acid degree values may be obtained when the solvents are added to the sample at the same time than when added separately. That is, when the solvents were in the ratio of 1:1 and added together to the sample, the average acid degree of the fat was 9.82, as compared to 9.65 when the solvents were added separately. The average acid degree of the fat obtained by churning the same cream was 7.45, a value 32 per cent lower than that of the fat extracted using the 1:1 ratio of extractants. In addition, the fat recovery was much less by the churning method, averaging about 70 per cent. On the basis of these observations, the procedure for fur-

ther trials consisted of adding the solvents at the same time and in the ratio of 2:3 (80 ml. of ethyl ether to 120 ml. of Skellysolve).

Observations of available data reveal that from the uniformity standpoint, variations in acid degrees between fat samples obtained by duplicate extractions or churning were similar. For example, average acid degree differences between duplicates for rancid fat were 0.114 when the fat was obtained by extraction and 0.171 when it was obtained by churning.

Effect of lactic acid and/or formalin. Since in certain cases formalin may be

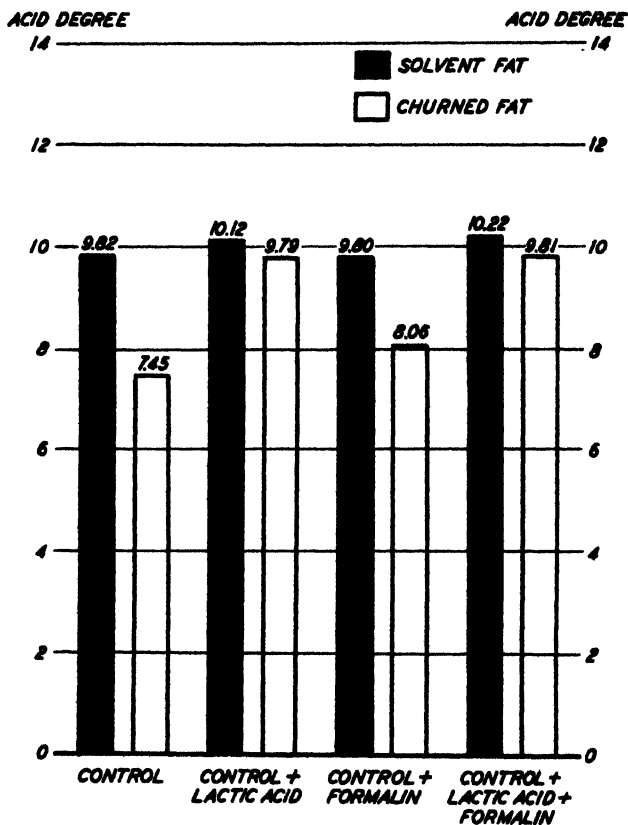


FIG. 2. Effect of lactic acid and/or formalin in the cream upon the acid degree of milk fat obtained by solvent extraction and churning methods.

added to the milk as a preservative, or in other cases lactic acid may be present due to bacterial action, the effect of these two substances upon the extraction and churning procedures and upon the free acid content of the fats was studied. In this study, rancid cream containing 50 per cent butterfat was divided into four lots and treated as follows: lot 1, control; lot 2, control + lactic acid (to give a concentration of 0.2 to 0.3 per cent); lot 3, control + formalin (1 ml./lb. of cream); lot 4, control + lactic acid + formalin (each added as above). Following this

treatment, fat samples were obtained from the various lots of cream by extraction and by churning and fat titrations were conducted. Results are illustrated in figure 2.

The figure shows that the addition of lactic acid and formalin had a marked effect upon the acid degree of fat obtained by churning but only a slight effect upon the acid degree of fat obtained by solvent extraction. The lactic acid addition produced an increase of 31.4 per cent in the acidity of churned fat, as compared to an increase of 3.1 per cent in the acidity of solvent fat. The same general trend, but to a lesser degree, resulted when formalin was present in the cream extracted or churned. In this instance, the acid degree of the resulting churned fat increased 8.2 per cent, whereas that of the solvent fat showed no appreciable change. When both lactic acid and formalin were added to cream prior to extraction or churning, the results were similar to those obtained when lactic acid alone was added, there being an increase of 31.7 per cent in acid degree in the churned fat, whereas that in the solvent fat was only 4.1 per cent.

TABLE 1
Recovery of butyric, caproic, capric and oleic acids from cream by solvent and churning methods

Trial No.	Butyric		Caproic		Capric		Oleic	
	Solv. ^a	Ch. ^b	Solv.	Ch.	Solv.	Ch.	Solv.	Ch.
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1			36.13	23.75				
2	4.03	1.44	14.42	13.11			92.36	88.28
3			42.05	6.27			104.20	83.77
4	9.33	0.62	24.20	10.70			71.40	77.10
5	6.59	1.69	25.40	7.99	76.1	84.8	90.25	80.40
Av.	6.65	1.25	28.44	12.36	76.1	84.8	87.05	82.36

^a Solvent method.

^b Churned method.

Churning was very difficult in the homogenized and rancid product and was made more so by the presence of formalin. Less than 50 per cent of the fat was recovered in such cases, whereas recovery by the solvent process was affected only slightly by such treatment of the cream.

Recovery of fatty acids from cream. The efficiency with which pure fatty acids are recovered from cream by solvent extraction and churning was studied. Butyric, caproic, capric and oleic acids were used. Approximately 0.25 *N* solutions of these acids were standardized accurately and added to good quality 30 per cent cream at the rate of 5 ml. of acid per 100 g. of cream. Fat was obtained from cream with and without added fatty acid by solvent extraction and by churning. Acid degree determinations were made and percentage recovery of the acids calculated. These data are presented in table 1.

Results reveal that the degree of recovery of the acids increased with increasing molecular weight of the acid. The solvent method gave somewhat better results with butyric and caproic acids and both methods were approximately equally efficient when capric and oleic acids were recovered.

Temperature and pressure of solvent removal. As described in the procedure, the general method for removing solvents was by heating the ethereal solution on a hot plate at 135° C., with final heating at 100° C. at a pressure of 500 mm. mercury. Since poor recoveries were obtained of the lower fatty acids, as revealed in table 1, it appeared desirable to determine the possibility of improving this recovery through the use of lower temperature and pressure for removing the solvents from the fat. Preliminary trials were conducted in which pure fatty acids were added to fat dissolved in ethyl ether and Skellysolve. The solvents were removed at (a) 100° C. and a pressure of 500 mm. mercury and (b) 60° C. and 20 mm. mercury. Recovery of butyric acid was increased from 22 to 80 per cent and that of caprylic from 80 to 100 per cent by reducing the temperature and pressure of solvent removal from the higher to lower levels. Loss of oleic acid was negligible in either case. On the basis of this work, the recovery of butyric acid from cream was studied, utilizing the solvent removal temperatures and pressures indicated above. Recovery of free butyric acid from cream was improved by lowering the pressure and temperature of solvent removal, with the recovery increasing from 12.2 per cent for the high temperature-high pressure procedure to 22.5 per cent for the low temperature-low pressure method. Since butyric acid comprises less than 4 per cent of milk fat, loss of even all of this acid which may have been freed by lipase action may not have any determinable effect upon the acid degree of a fat sample from rancid cream.

The improvement in the recovery of lower fatty acids added to cream by the use of lower temperatures and pressures raises the question as to whether or not such conditions would increase the recovery of those free fatty acids produced normally in milk by lipase action. To study this possibility, several trials were conducted in which fat samples from normal and rancid creams were obtained by solvent extraction and by churning with the solvent-extracted fat being dried under the two conditions of temperature and pressure. The results in acid degrees were 0.853 and 0.875 for normal cream, 10.262 and 10.267 for rancid cream for the high temperature and low temperature treatment, respectively. Therefore, these data reveal no appreciable differences between the acid degrees of the extracted fats obtained and, thus, no advantage in favor of the lower temperature is indicated.

Flavor of extracted and churned fat. In connection with various phases of this study, flavor observations were made on the fat obtained by churning and by extraction. Results for some of these observations are presented in table 2.

These limited data indicate that no churned fats were rancid, whereas a number of the extracted fats from rancid milk showed appreciably rancid flavors which were characterized as butyric, caprylic, goaty or bitter. It also was noted that temperature and pressure of solvent removal affected the flavor of the fat. When the temperature and pressure were 60° C. and 20 mm. mercury, respectively, the fat more often was butyric- or caprylic-flavored. However, when the solvents were removed at 100° C. and 500 mm. mercury, goaty or bitter flavors predominated in the resulting fat.

Analysis of churned and extracted fats. To determine the chemical differences between the fat isolated by churning and by extraction, fat samples obtained

TABLE 2
Effect of method for obtaining fat on its flavor

Method for obtaining fat	Flavor of cream	Flavor and incidence of flavor in fat
Churning	Normal Rancid	Oxidized, 2 ^a Oxidized, 5
Solvent extraction	Normal Rancid	Oxidized, 5; solvent, 4; Butyric, 4; caprylic, 4; Goaty, 4; ester, 2; solvent 3.

^a Figure indicates flavor intensity, 1 being minimum; 5, maximum.

from normal (control) and rancid cream by the two procedures were subjected to analysis. In these trials, the ethereal solution in the solvent method was evaporated at 60° C. and 20 mm. mercury. Results obtained are presented in table 3. These data reveal that, with the exception of the acid degree values, such determinations fail to show any appreciable difference between solvent and churned fats and between rancid and non-rancid fats. The Reichert-Meissl values gave slightly, but consistently, lower values for the rancid fats in comparison to the control fats. Differences in the Polenske and saponification values and in iodine numbers are insignificant.

The acid degree determinations on these fats gave the only results which show that appreciable differences exist between rancid and control samples and between extracted and churned fats. For the churned fat, there was a 17-fold difference in acid degree between the normal and rancid samples (0.46 and 7.95) and for the extracted fat the difference was greater than 18-fold (0.77 and 13.94, respec-

TABLE 3
Chemical composition of solvent-extracted and churned fats

Fat characteristic	Trial	Churned fat		Solvent fat	
		Control	Rancid	Control	Rancid
Reichert-Meissel value	4	28.54	27.78	28.51	27.39
	14	27.36	27.17	27.42	26.72
	21	29.77	29.54	29.79	28.84
	Av.	28.56	28.16	28.57	27.65
Polenske value	4	1.60	1.65	1.70	1.64
	14	2.10	2.09	2.09	1.99
	21	2.27	2.21	2.39	2.30
	Av.	1.99	1.98	2.06	1.98
Iodine value	4	43.10	42.93	43.28	42.75
	14	37.82	38.14	37.85	37.62
	21	33.98	34.31	34.05	34.09
	Av.	38.30	38.46	38.39	38.15
Saponification value	4	226.6	225.5	225.5	226.8
	14	222.4	223.0	223.7	221.5
	21	228.4	227.2	226.5	226.5
	Av.	225.8	225.2	225.2	224.9
Acid degree	4	0.64	7.19	0.925	15.375
	14	0.38	6.53	0.72	13.950
	21	0.357	10.115	0.661	12.505
	Av.	0.459	7.945	0.769	13.943

tively). The solvent-obtained fat in all cases yielded higher acid degrees than did the respective churned fats. The rancid solvent fat was 75 per cent greater in acid degree than that obtained by churning, and the control solvent fat exhibited 68 per cent greater acidity than did the churned fat.

Ethanol extraction of fat. In the first paper of this series, an ethanol extraction procedure is described which permits a high concentration of the free fatty acids present in the fat (3). Since the previous study was of churned fat it seemed desirable to subject the solvent extracted fat to this ethanol fractionation to ascertain if it would reveal more information as to the fatty acids involved in lipase action.

Milk fat obtained by the solvent method from non-rancid and rancid cream was fractionated by extracting 60 g. of fat in three successive portions of boiling ethanol, and then cooling and filtering the extract. Three fractions were obtained. I. Ethanol-insoluble fat, (portion remaining after extraction); II. Cold

TABLE 4
Chemical characteristics of the fractions obtained by extraction of control and rancid fats with ethanol (three trials)

Fraction analysed	Acid degree	Reichert-Meissl value	Polenske value	Saponification value	Iodine value	Refractive index	Wt. obtained/100 g. original fat
							(g.)
Original fat							
Control	0.74	26.87	1.86	222.4	40.25	1.4546	...
Rancid	13.41	25.40	1.88	221.5	38.65	1.4542	...
Cold ethanol extract							
Control	9.06	49.09	4.55	237.2	42.73	1.4551	6.33
Rancid	78.14	37.89	3.01	225.8	36.91	1.4526	13.51
Cold ethanol precipitate							
Control	0.79	41.60	2.32	235.1	32.14	1.4530	7.90
Rancid	5.19	38.24	2.34	232.1	30.29	1.4528	8.83
Ethanol insoluble fat							
Control	0.15	21.79	1.74	218.2	40.34	1.4551	82.30
Rancid	1.37	19.56	1.39	215.1	39.67	1.4547	73.29

ethanol precipitate, (that portion which precipitated from the ethanol after it stood overnight at 4-5° C. and which was removed by filtration); III. Cold ethanol extract (portion remaining in solution in the ethanol after cooling, i.e., the filtrate of II). The average weights of these various fractions obtained from 100 g. of normal milk fat were 82.3 g. of ethanol-insoluble fat, 7.9 g. of cold ethanol precipitate and 6.3 g. of cold ethanol extract. From 100 g. of rancid fat these weights were 73.3 g., 8.8 g. and 13.5 g., respectively. Chemical analysis of these fractions and of the original fat are shown in table 4.

These data reveal, as was found previously (3), that the free fatty acids in fat may be concentrated by alcohol extraction. The acid values of the alcohol extracts show approximately a 12-fold increase in the case of the control product and a 6-fold increase in the rancid product, when compared to the corresponding original fat. In contrast, the cold ethanol precipitate contained about the same amount of free acidity as the original fat in the control and less acidity in the rancid product. The acid degrees of 0.15 and 1.37 for the ethanol-insoluble fat

for the control and rancid samples, respectively, indicate that the alcohol extraction removes the major portion of the free fatty acids from milk fat.

Reichert-Meissl values of the various fractions from non-rancid and rancid fat show that the volatile soluble fatty acids are concentrated in the cold ethanol extract and precipitate. As expected, the ethanol-insoluble fat contained fewer of these short-chain acids. The volatile insoluble fatty acids also were concentrated in the ethanol extract and precipitate, as shown by the Polenske values.

The results obtained by the Reichert-Meissl and Polenske determinations are substantiated by the saponification values, which again are higher for the ethanol extract and precipitate and lower for the ethanol-insoluble fat, as compared to the original fat.

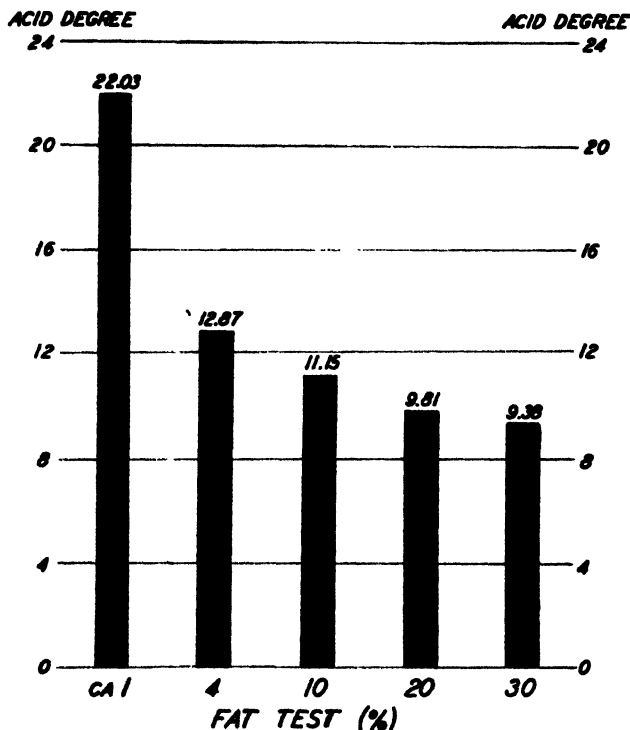


FIG. 3. Relationship between the fat content of the product and the acid degree of the fat obtained by extraction.

The iodine values obtained show no definite trend, except that they were lower for the ethanol precipitate than for the other fractions.

The refractive indices reflect roughly the values obtained by the other determinations. For instance, the large differences in refractive indices between the control and rancid ethanol extracts may be explained by the lower content of unsaturated fat and the greater amount of free fatty acid in the latter.

Application of the solvent extraction procedure to milk. Since the procedure in the foregoing studies involved the separation of rancid milk to obtain

the cream for subsequent extraction or churning, it was thought that an appreciable amount of the free fatty acids, especially the water-soluble acids, may be lost in the skim milk. To ascertain this effect, trials were conducted in which the extraction procedure was applied to milk and cream varying widely in fat content. Raw milk was homogenized, allowed to become rancid and separated to 30 per cent cream. Portions of this cream were standardized to 4, 10 and 20 per cent fat with the skim milk. Due to homogenization, separation was inefficient and about one per cent fat remained in the skim milk. All of these products were extracted and the acid degrees of the extracts determined. The data obtained are portrayed in figure 3.

This figure shows that the acid degree of the fat decreases with increases in the fat content of the product extracted. The value of 22.03 for the skim milk extract is nearly twice that of 12.87 for 4 per cent milk, and further decreases in fat acidity are apparent as the fat content of the products increases to 30 per cent. The fat extracted from the 4 per cent milk, which may be considered to be the original rancid milk before separation, had more than 36 per cent greater acidity than the fat from the 30 per cent cream, the only difference in the two products extracted being the amount of skim milk present.

DISCUSSION

In past work, little attention was paid to the method for isolation of the milk fat in lipase studies. The fat was obtained merely in the quickest and most convenient manner, usually by churning, without special regard for fatty acids which might be lost through such treatment. The results obtained in this study reveal the advantages of fat recovery by solvent extraction rather than by churning. Not only does the solvent method result in more complete recovery of the fatty acids, as revealed by the higher acid degree of the fat, but the results are affected less by the presence of such substances as lactic acid and formalin. Thus, the procedure may be utilized successfully on a fermented product or on a preserved product. Such is not the case with the churning procedure, where the values are affected appreciably by the presence of either lactic acid or formalin. A further advantage of the extraction method is its use in the isolation of lipids for titration from homogenized products of low fat content or from milk homogenized at high pressures.

Many studies have been conducted in which the titer of fat obtained by churning has served as the basis of comparison. For example, threshold values for flavor have been suggested (2, 5). Also, results have been obtained in which acidity changes in fat obtained by churning have been correlated with production and transportation methods for raw milk (5, 6). On the basis of the results herein reported, the need for a re-evaluation of all studies made when the fat was obtained by churning is indicated.

Further evidence of the greater efficiency of the extraction procedure is given by the fact that rancidity was detected in solvent-extracted fat from rancid milk, whereas churned fat from the same source did not exhibit a rancid odor. That the flavor of several samples was typical of the "goat" acids rather than of butyric acid indicates that the free caproic, caprylic and capric acids are

recovered more efficiently by the extraction procedure than is the more water-soluble butyric acid. This is borne out by the fact that the best recovery of butyric acid from cream was only about 23 per cent. Lack of rancid odor in churned fat may be expected since negligible recovery of butyric and caproic acids results from the churning procedure. The low recovery of butyric acid in solvent extraction may be attributed chiefly to its inefficient extraction from the serum phase, since a rather drastic method of solvent removal was shown to account for only a 20 per cent loss of the acid added to a solution of butterfat. The distribution ratios for butyric acid between ethyl ether and water are involved in this connection. These ratios (ether to water) vary from 4.2:1 to 5.3:1 for concentrations of the acid from 0.01 per cent to 0.12 per cent, respectively (10), values which would account at least partially for losses during the actual extraction.

The application of the extraction procedure to low-fat products or to homogenized products further emphasizes the weakness of the churning method. The data reveal the effect of separation on the loss of ether-extractable acids, losses which would not be detected when the fat is isolated by churning. The loss of these acids during separation, in addition to those lost if the cream were churned rather than solvent-extracted, would result in recovery of less than one-half the free fatty acids obtainable by direct extraction of the milk.

Chemical analysis of churned and solvent fat failed to show any appreciable differences between these fats, whether normal or rancid, except through comparison of acid degrees. These data agree with previous results based on fat obtained by churning (4). The data presented serve to illustrate that only a small portion of the fat is affected by lipolysis. An average saponification number of 225 is equivalent to an acid degree of 402 if the fat is totally hydrolyzed. On this basis, only 1.97 per cent of the churned rancid and 3.46 per cent of the solvent rancid fat was hydrolyzed. Therefore, small losses of free fatty acids, through either solvent extraction or churning, would have no appreciable effect upon the characteristics of the fat obtained.

Analysis of the various fractions resulting from ethanol extraction of solvent-extracted milk fat gave no definite indication of selective fat hydrolysis by the lipase system. However, as indicated previously (3), the free fatty acids may be concentrated by such extraction. It is evident that not only the free fatty acids are extracted but also any mono-, di-, and triglycerides which may be soluble in the hot ethanol. The precipitate formed upon chilling of this solution is comparatively low in free acids and high in Reichert-Meissl, Polenske and saponification values, indicating that glycerides of shorter-chain acids are involved. The cold alcohol-soluble fraction contained the bulk of the fatty acids, and probably mono- and diglycerides of the lower fatty acids, as indicated by high acid degree, Reichert-Meissl, Polenske and saponification values.

SUMMARY

A method for the removal of fat from milk products which involves the use of ethanol, ethyl ether and Skellysolve F was developed.

The application of this solvent extraction method to rancid milk yielded fat averaging 30 per cent higher in acidity than fat obtained by churning and re-

sulted in better recovery of the fat. The solvent method is adapted particularly to the removal of fat from homogenized products of low fat content, thereby eliminating the loss of water-soluble fatty acids through separation. Moreover, the addition of lactic acid and/or formalin to cream had little effect upon the acidity of the fat resulting from solvent extraction, but increased appreciably the acidity of that obtained by the churning process.

The solvent method was superior to the churning method for recovery of pure fatty acids from cream, particularly the lower acids, butyric and caproic. This is emphasized by the fact that many samples of fat which were solvent-extracted from milk exhibited a rancid flavor, whereas churned fat from the same source did not. However, even with the solvent method, butyric acid recovery was low as determined by titration.

Improved recovery of butyric acid added to cream resulted when the temperature and pressure of solvent removal from fat was lowered from 100° C. and 500 mm. mercury to 60° C. and 20 mm. mercury. However, when rancid cream was extracted, these modifications proved to be of questionable value so far as fat titration values were concerned, due to the small amounts of volatile, water-soluble fatty acids present.

Chemical analysis failed to show any appreciable differences, other than in acid degree, between solvent-extracted and churned fat, whether the fat was from normal or rancid milk. Also, analysis of the ethanolic soluble and insoluble fractions of solvent-extracted fat failed to indicate definitely selective hydrolysis by milk lipase.

The data presented in this study indicate the need for re-evaluation of results from lipase studies which are based upon the titration of fat obtained by churning methods.

REFERENCES

- (1) ASSOC. OF OFFICIAL AGR. CHEMISTS. Official and Tentative Methods of Analysis, 4th Ed. 1935.
- (2) GOULD, I. A. Relationship of Fat Acidity to Rancidity in Homogenized Raw Milk. *J. Dairy Sci.*, 27: 167-172. 1944.
- (3) GOULD, I. A. Milk Lipase System. I. Chemical Analysis of Fat, Washed Fat, and the Alcohol Soluble Fraction of Fat Obtained from Homogenized Raw Milk. *J. Dairy Sci.*, 30: 901-907. 1947.
- (4) GOULD, I. A., AND TROUT, G. M. The Effect of Homogenization on Some of the Characteristics of Milk Fat. *J. Agr. Research* 52: 49-57. 1936.
- (5) HERRINGTON, B. L., AND KRUKOVSKY, V. N. Studies of Lipase Action. I. Lipase Action in Normal Milk. *J. Dairy Sci.*, 22: 127-135. 1939.
- (6) HLYNKA, I., HOOD, E. G., AND GIBSON, C. A. Agitation and Temperature of Cheese Milk and the Development of Rancid and Unclean Flavors in Cheddar Cheese. *J. Dairy Sci.*, 26: 1111-1119. 1943.
- (7) JACK, E. L., TARASSUK, N. P., AND SCARAMELLA, E. L. Effect of Different Pasteurization Temperatures upon Keeping Quality of Butter Made from Cream Containing Active Lipase. *Natl. Butter Cheese J.*, 33, 12: 16-20. 1942.
- (8) JOHNSON, B. C., AND GOULD, I. A. Variations in the Acid Degree of Milk Fat as Affected by Churning and Extraction Procedures. *J. Dairy Sci.*, 29: 504-505. 1946.
- (9) KRUKOVSKY, V. N., AND HERRINGTON, B. L. Studies of Lipase Action. VI. The Effect of Lipolysis upon the Flavor of Milk. *J. Dairy Sci.*, 25: 237-239. 1942.
- (10) NATIONAL RESEARCH COUNCIL. International Critical Tables. McGraw-Hill Book Co., New York. 1926.

MILK LIPASE SYSTEM. III. FURTHER STUDIES OF THE SOLVENT EXTRACTION PROCEDURE FOR OBTAINING FAT FROM MILK FOR TITRATION¹

B. C. JOHNSON AND I. A. GOULD²

Maryland Agricultural Experiment Station, College Park

In previous work (5) a solvent extraction procedure was developed which proved superior to standard churning methods for the obtaining of fat in milk lipase studies. However, even this solvent method was found to be inefficient in removing butyric and caproic acids from cream. Thus, it seemed desirable to modify the extraction method to obtain greater recovery of the fatty acids resulting from milk lipase activity. The results of such modifications are presented in this paper. In addition, a comparison was made between the extraction procedure as developed, and a continuous extraction method used by Kelly (6, 7) to obtain fat for titration.

METHODS

The general conditions of the experiment were as described in the preceding paper (5). Fresh, raw milk was obtained from the University herd and separated to a 10 per cent product. The milk was homogenized by a rotary machine to accelerate the lipase activity. Samples were incubated at 37° C. with formalin (1 ml. per lb. of milk) as preservative. Lipase action was stopped by heating the milk to 65° C. or above for 10 minutes.

The fat was obtained for titration either by the solvent extraction procedure or by a continuous extraction method adapted from Kelly's work (7). In the solvent extraction method, 125 g. of cream were shaken vigorously 15 seconds with 100 ml. of ethanol and allowed to stand for 5 minutes. Then 80 ml. of ethyl ether and 120 ml. of Skellysolve³ were added and the mixture was shaken again for 30 seconds. The emulsion was broken by centrifugation and the ethereal layer removed. The fat was freed of solvents at 60° C. and 20 to 24 mm. mercury. The solvent extraction procedure was modified in two ways. In the first modification, the cream or milk was saturated with either NaCl or MgSO₄ before the extraction was conducted. In the second modification, the cream or milk was adjusted to pH 2 with H₂SO₄ (1 + 3), using rapid agitation, prior to extraction.

In the continuous extraction method, the sample was mixed with two to three times its weight of plaster of Paris, allowed to harden overnight and then extracted with ethyl ether in a Soxhlet apparatus. A 1,000-ml. size extractor was used, requiring 4.5 to 5 minutes per cycle. Extraction was carried out for at

Received for publication January 10, 1949.

¹Scientific paper no. A225. Contribution no. 2150 of the Maryland Agricultural Experiment Station (Dairy Department).

²Present address: Dairy Technology Dept., Ohio State University, Columbus.

³Skellysolve F—a petroleum ether, boiling range 30 to 60° C.

least 3.5 hours per sample. Under these conditions, recovery of the fat was 95 per cent or better.

In previous work (6), fat titrations were made by the A.O.A.C. method (1). Although this method was found to give acceptable results, some difficulty was experienced with the endpoint, especially in highly rancid samples. This matter of titration was investigated and comparisons made among four methods, those of Breazeale and Bird (2), Herrington and Krukovsky (4), Clarke *et al.* (3) and A.O.A.C. (1). The results were comparable for all methods. However, the alcoholic KOH method of Breazeale and Bird was chosen for this work because the titration was in a clear solution and the endpoints were the most definite and exhibited the least fading. Titration results were expressed in acid degrees (the number of ml. of *N* alkali required to neutralize the acids in 100 g. of fat).

In studies on recovery of pure fatty acids, the acids were standardized accurately at approximately 0.25 *N* and added to 30 per cent cream at the rate of 5 ml. per 100 g. or to 10 per cent milk at the rate of 2 ml. per 100 g.

EXPERIMENTAL

Saturation with Neutral Salts. In the first phase of this work, the effect of saturation of the cream with either NaCl or MgSO₄ prior to extraction on the re-

TABLE 1
Effect of salt-saturation of the cream upon the recovery of butyric acid

Treatment of the Cream	Recovery of Butyric Acid		
	Trial 1	Trial 2	Av.
	(%)	(%)	(%)
Control (cream plus butyric acid)	19.0	9.48	14.24
Control plus NaCl	31.5	18.96	25.23
Control plus MgSO ₄	23.5	8.03	15.77

covery of butyric acid from 30 per cent cream was determined. Results are presented in table 1. These data reveal that saturation of cream with NaCl tended to improve recovery of butyric acid, whereas saturation with MgSO₄ did not appreciably affect recovery of the acid. Addition of NaCl increased recovery of butyric acid by 11 per cent.

In view of these results, studies were conducted on normal and rancid milk (10 per cent) in which the fat was obtained by solvent extraction with and without the aid of NaCl saturation. The results presented in table 2 reveal that the acid degree of the fat obtained from both normal and rancid products was not affected appreciably by saturation of the milk with the salt, although the results were inconsistent between trials.

Acidification with H₂SO₄. In studies involving the effect of lowering the pH the recoveries of butyric, caproic, capric and oleic acids from acidified and untreated 10 per cent milk were compared. Results are presented in table 3. Acidification of the milk with H₂SO₄ was found to increase appreciably the re-

TABLE 2

Effect of NaCl-saturation of the milk before extraction upon the acid degree of the fat

	Acid Degree of the fat			
	Trial 1	Trial 2	Trial 3	Av.
	(%)	(%)	(%)	(%)
Without NaCl				
Normal fat	1.13	1.22	0.87	1.07
Rancid fat	13.59	27.03	15.84	18.82
With NaCl				
Normal fat	1.04	0.67	0.70	0.80
Rancid fat	13.70	27.16	15.46	18.77

covery of various fatty acids from 10 per cent milk. The greatest increases were noted with butyric and caproic acids. Recovery of the former was more than tripled (9.48 to 30.59 per cent) and that of the latter nearly doubled (42.39 to 72.26 per cent). Total recovery of caprylic and oleic acids was achieved when the pH of the milk was adjusted prior to extraction.

TABLE 3

Effect of acidification of milk before extraction upon the recoveries of added fatty acids

Fatty acid added	Acid degree			Recovery of pure fatty acid	
	Calculated increase	Actual increase			
		Not acidified	Acidified	Not acidified	Acidified
				(%)	(%)
Butyric	6.13	0.58	1.87	9.48	30.59
Caproic	5.57	2.36	4.02	42.39	72.26
Capric	5.32	4.46	5.43	83.79	102.20
Oleic	4.61	4.17	4.73	90.45	102.80

The effect of acidification of normal and rancid 10 per cent milks upon the acid degree of the extracted fats is shown in table 4. In all cases, the acid degrees of the fats from acidulated creams were higher, averaging 1.38 and 19.79 for normal and rancid acid-treated samples, as compared to 1.07 and 18.82, respectively, for the untreated samples. Since qualitative tests for the sulfate ion were negative, the increase could not be attributed to entrained H_2SO_4 .

TABLE 4

Effect of acidification of the milk before extraction upon the acid degree of the fat

	Acid degree of the fat			
	Trial 1	Trial 2	Trial 3	Av.
	(%)	(%)	(%)	(%)
Cream not acidified				
Normal fat	1.13	1.22	0.87	1.07
Rancid fat	13.59	27.03	15.84	18.82
Cream acidified with H_2SO_4				
Normal fat	1.37	1.62	1.16	1.38
Rancid fat	14.13	28.49	16.73	19.79

Comparison with Continuous Extraction Method. The data obtained when the standard solvent extraction method was used were compared to those which resulted from the continuous extraction of milk dried with plaster of Paris. Results are presented in table 5. Continuous extraction of dried milk, whether normal or rancid, produces fat of much lower acidity than does standard solvent extraction. When the milk was not acidified prior to extraction, the average differences in acid degree between fats from rancid and normal milks were 8.24 for the former method and 17.75 for the latter, or a difference of 115 per cent. Acidification of milk prior to extraction increased the acidity of fat obtained by standard extraction to a lesser extent than that of fat obtained by continuous extraction; however, the acid degree obtained by the former method was still

TABLE 5

A comparison of the acid degree of fat obtained by solvent extraction and by continuous extraction of milk previously dried with plaster of Paris

	Acid degree of the fat when the cream was:	
	Not acidified	Acidified
Solvent extraction method		
Normal	1.07	1.38
Rancid	18.82	19.79
Difference	17.75	18.41
Continuous extraction method		
Normal	0.78	0.87
Rancid	9.02	12.84
Difference	8.24	11.97

54 per cent greater than that by the latter - 18.41 as compared to 11.97. Percentage recovery of the fat was satisfactory by either method.

DISCUSSION

Since butyric acid is the most volatile and water-soluble fatty acid in butterfat, the problem of recovery of all of the fatty acids present becomes one of obtaining more complete recovery of this acid. Substances which are appreciably soluble in water often may be extracted more successfully with ether if an inorganic salt is first added to the solution to reduce the solubility of the substance in the water. Although NaCl was found to increase the extraction of butyric acid to some extent, the improvement in butyric acid recovery by saturation of the milk with NaCl does not appear sufficiently great to warrant its use; the effect is slight and inconsistent when based on the acid degree of the fat from rancid and non-rancid milk. Saturation of cream with $MgSO_4$ did not improve butyric acid recovery.

The improved recovery of butyric, caproic, capric and oleic acids which resulted when the milk was adjusted to pH 2 prior to extraction is sufficient to justify the acceptance of such treatment as a part of the solvent extraction procedure. In addition, when this method was used with rancid cream, the acid degree of the resulting fat was appreciably higher than when the fat was extracted without pH adjustment. Apparently, under normal conditions, an ap-

preciable portion of the free fatty acids is bound in the milk, probably as salts, and thus is not removed by ordinary solvent extraction.

The results obtained in this work raise considerable question about the accuracy of the continuous extraction of milk which has been dried with plaster of Paris. Even with excessively long extraction times, the acid degree of fat obtained by continuous extraction was less than one-half that obtained by the standard solvent procedure and the results lacked consistency.

SUMMARY

1. Results are presented of two attempts to modify the solvent extraction procedure for the obtaining of fat in lipase studies so as to increase the efficiency of recovery of the lower fatty acids. The two modifications studied were: (a) the saturation of the milk with salts (MgSO_4 and NaCl) before extraction and (b) the acidification of the milk to pH 2 with H_2SO_4 before extraction.

2. Of these modifications, only the acid adjustment of the milk or cream was found to yield sufficient improvement in recovery as to warrant its adoption as a part of the solvent extraction procedure.

3. A comparison of the solvent extraction method and a procedure involving continuous extraction of milk previously dried in plaster of Paris reveals that the latter method does not remove efficiently the free fatty acids from milk.

REFERENCES

- (1) ASSOC. OF OFFICIAL AGR. CHEMISTS. Official and Tentative Methods of Analysis. 4th ed. 1935.
- (2) BREAZEALE, D. F., AND BIRD, E. W. A Study of Methods for the Determination of Acidity in Butterfat. *J. Dairy Sci.*, 21: 335-344. 1938.
- (3) CLARKE, J. O., CANNON, J. H., COULTER, E. W., GOODMAN, M. S., GREENE, W. S., MILSTEAD, K. L., VANDAVEER, R. L., AND WILDMAN, J. D. Detection of Decomposition Products in Butter and Cream. *J. Assoc. Official Agr. Chemists*, 20:475-505. 1935.
- (4) HERRINGTON, B. L., AND KRUKOVSKY, V. N. Studies of Lipase Action. I. Lipase Action in Normal Milk. *J. Dairy Sci.*, 22: 127-135. 1939.
- (5) JOHNSON, B. C., AND GOULD, I. A. Milk Lipase System. II. Comparison of Solvent Extraction and Churning Methods for Obtaining Fat From Milk for Free Fatty Acid Measurement. *J. Dairy Sci.*, 31: 435-446. 1949.
- (6) KELLY, P. L. The Lipolytic Activity of Bovine Mammary Gland Tissue. *J. Dairy Sci.*, 26: 385-399. 1943.
- (7) KELLY, P. L. Milk Lipase Activity: A Method for its Determination and its Relationship to the Estrual Cycle. *J. Dairy Sci.*, 28: 803-819. 1945.

A COMPARISON OF TORSION PENDULUM TYPE VISCOSIMETERS FOR MEASUREMENT OF VISCOSITY IN DAIRY PRODUCTS¹

F. E. POTTER, E. F. DEYSHER, AND B. H. WEBB
*Bureau of Dairy Industry, Agricultural Research Administration,
U. S. Department of Agriculture*

In conjunction with studies on the viscosity of dairy products it was necessary to examine various viscosimeters to determine their accuracy and operating characteristics. The purpose of this article is to show some of the difficulties encountered in determining the viscosity of dairy products and to present conversion factors, for torsion pendulum viscosimeters, which may be of use to others in similar studies.

Whitaker and Herrington (4), using the Mojonnier-Doolittle viscosimeter, developed an equation for the conversion of degrees retardation to absolute viscosity. Herschel (1, 2), studying the MacMichael viscosimeter, found the constants (factors) obtained with liquids of a known viscosity to be nearly identical with the calculated constants for wires of 26, 30 and 34 gauge. However, the 34 and 36 wires gave nearly identical results and the constant for the 36 wire was considerably larger than the calculated constant. He concluded that this wire was of larger diameter than the standard for that gauge. Sheely (3) reported that the values obtained with two of the pipet type viscosimeters agreed very closely on liquids of known viscosity, whereas the MacMichael gave slightly higher values. This difference increased with an increase in viscosity.

EXPERIMENTAL PROCEDURE

In this study three viscosimeters of the torsion pendulum type were used, namely the Brookfield,² the MacMichael,³ and the Mojonnier-Doolittle.⁴ The operation of the instruments was conducted as specified by the manufacturers. The Brookfield viscosimeter was used with four attachable spindles or plungers, and operated at speeds of 6, 12, 30 and 60 revolutions per minute. Wires of a standard gauge numbering from 18 to 30, inclusive, with both large and small plungers, were used with the MacMichael viscosimeter. The three interchangeable plungers provided with the Mojonnier-Doolittle viscosimeter were used with three wires of the same size.

All determinations in this study were conducted at 86° F. (30° C.). A separate portion of the sample was used for each determination to eliminate any error caused by structural breakdown due to the action of the plungers.

The depth of plunger immersion in the product to be tested was uniform for each viscosimeter. The Brookfield spindles were immersed to the indentation on

Received for publication January 11, 1949.

¹ This work was done in part with funds made available through the Agricultural Research and Marketing Act of 1946.

² Brookfield Engineering Laboratories Inc., Stoughton, Massachusetts.

³ Eimer and Amend, New York, N. Y.

⁴ Mojonnier Bros. Co., Chicago, Illinois.

TABLE 1

Mojonnier-Doolittle viscosimeter conversion factors^a obtained with oils of known viscosities

Standard oils		Wire no.	Size of plunger					
Oil no.	Viscosity at 86° F.		Small		Medium		Large	
			Reading ^b	Factor	Reading ^b	Factor	Reading ^b	Factor
<i>centipoises</i>								
M -14	148.3	1	8.33	17.8	60.66	2.44	224.66	0.660
M -14	148.3	2	8.00	18.5	59.66	2.48	209.66	0.707
M -13	155.0	1	5.33	29.0	58.59	2.64	219.17	0.707
M -13	155.0	2	7.33	21.1	59.36	2.61	214.93	0.721
M -13	155.0	3			55.50	2.79	211.00	0.735
N -15	648.0	1	18.66	34.7	197.33	3.28		
N -15	648.0	2	24.66	26.2	201.66	3.21		
OB- 3	13,930.0	1	222.77	62.5				
OB- 3	13,930.0	2	224.69	61.9				
OB- 3	13,930.0	3	222.00	62.7				
Av. Factors				37.2	2.78		0.706	

^a Factor × reading = centipoises.^b Each reading represents an average of five or more determinations.

the spindle shaft; for the MacMichael, the topmost mark was used for the small plunger and the bottom of the small knob on the side of the cup for the large plunger. With the Mojonnier the level of the fluid was adjusted to cover exactly the bulb of the plunger.

Standard viscosity oils were obtained from the National Bureau of Standards for instrument standardization. The viscosity of these oils at 86° F. is included in the data of table 1 with the exception of oil P8, which had a viscosity of 43,630 centipoises at 86° F.

RESULTS

In the standardization of the viscosimeters it was found that the conversion factors furnished with the Brookfield were satisfactory when tested with the standard oils.

The conversion factors obtained with the Mojonnier-Doolittle viscosimeter are presented in table 1. The data show only a slight variation in results obtained

TABLE 2

Factors for converting MacMichael viscosimeter readings taken at 86° F. to centipoises when the viscosimeter cup is turned at 21 r.p.m.

Wire	Factors ^a	
Standard Gauge no.	Small Plunger	Large Plunger
18	11,368	217
20	4,475	93
22	1,694	37
24	703	13.6
26	286	5.8
28	82	1.73
30	43	0.901

^a Factor × °M = Centipoise

with the different wires, and this variation probably is within the accuracy of the determination. The factors for the small plunger varied from 17.8 to 62.7, the medium plunger 2.44 to 3.28, and the large plunger 0.660 to 0.735, with average factors of 37.2, 2.78, and 0.706, respectively.

Table 2 shows the factors obtained in the standardization of the MacMichael viscosimeter. These data represent the average of several trials with two or more oils for each wire, the individual determinations varying only slightly.

Figure 1 shows a straight line relationship between the logarithm of the factors and the wire or spindle size for both the MacMichael and Brookfield viscosimeters. A similar plot of the Mojonnier data gives a curve which indicates a somewhat different relationship between the logarithm of the factor and the plunger sizes of this instrument.

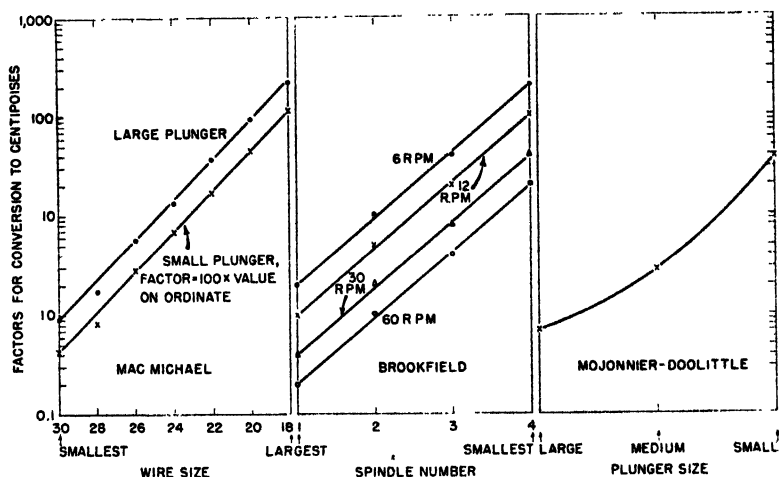


Fig. 1. The relationship between viscosity conversion factors and the wire or spindle sizes of three different viscosimeters.

Measurements next were made on various dairy products and the viscosities were calculated by using the factors determined for the different viscosimeters. Several determinations were made on each of a number of samples.

The data of table 3 show the variations in values obtained with the three viscosimeters, as well as differences found in the viscosity values when different wires or plungers were used with the same instrument. With the MacMichael viscosimeter, the viscosity decreased with an increase in the wire gauge for both the large and small plunger. Although this table does not show values obtained with other wires, the same relationship existed. In the results obtained with the Mojonnier-Doolittle viscosimeter, the data show a decrease in the viscosity value with an increase in the plunger size. For the Brookfield viscosimeter an increase in the revolutions per minute of the spindle caused a decrease in the viscosity value. In addition, in nearly all cases when operating at the same speed, the larger the spindle used, the smaller the viscosity value. This relationship was found in many other samples.

TABLE 3

Viscosity values of various dairy products determined with the three viscosimeters and converted to centipoises with the factors obtained for each instrument

Product	MacMichael viscosi-meter		Mojonnier viscosi-meter		Brookfield viscosimeter						Av. value
	Large plunger		Medium plunger	Large plunger	#1 Spindle ^a R.P.M. ^b				#2 Spindle R.P.M.		
	#28 wire	#30 wire			6	12	30	60	30	60	
Evaporated milk	(c.p.)	(c.p.)	(c.p.)	(c.p.)	(c.p.)	(c.p.)	(c.p.)	(c.p.)	(c.p.)	(c.p.)	(c.p.)
Condensed skim milk	42.8	40.9	55.6	53.0	48.0	49.0	43.9	39.4	48.0	40.7	46.1
Reconstituted skim milk (approx. 30% solids)	66.7	61.0	94.6	74.8	82.0	79.0	71.2	65.0	70.0	67.0	73.1
Ice cream mix ^c	51.3	47.9	58.4	57.2	54.0	51.0	49.2	47.5	58.0	50.3	52.5
	56.4	54.0	69.6	66.4	87.4	74.7	60.0	51.2	67.4	52.0	63.9
	Small plunger		Small plunger		#3 Spindle R.P.M.		#4 Spindle R.P.M.				
	#28 wire	#30 wire		6	12	6	12	30	60		
Sweetened condensed whole milk	2,654	2,616	2,235		3,040	2,840	3,060	2,870	2,600	2,460	2,708
Sweetened condensed skim milk	9,436	8,328	5,550		11,040	9,494	10,800	9,500	7,840	6,620	8,734

^a Number 1 spindle is the largest, number 4 is the smallest.

^b R.P.M. = revolutions per minute.

^c This sample of ice cream mix was abnormally low in viscosity.

DISCUSSION

It is well known that the usual viscosity values obtained on such products as ice cream mix and sweetened condensed milk are not absolute; they do not take into consideration plasticity effects and they must be run in the same manner each time if comparative figures are to be obtained. Nevertheless, relative viscosity determinations do give numerical values which reflect the treatment of products during manufacture and storage and which provide a means of comparison and of grading.

The factors presented here will be useful to workers wishing to make relative viscosity determinations on dairy products. It should be pointed out, however, that even though the appropriate conversion factors are used, the viscosity values will vary with the wire or plunger size as well as with the speed of rotation. The conversion factors furnished by the manufacturers of the Brookfield viscosimeter were found to be satisfactory. With this instrument the operating speed had a greater influence on the viscosity value of dairy products than did the spindle size.

In general, the faster the speed of rotation and the larger the spindle size, the lower the viscosity value.

Conversion factors for each MacMichael wire with both the large and small plungers checked very closely with all oils. A speed of 21 revolutions per minute for the MacMichael cup was used in this study, and operation at another speed would give a different set of factors for the respective wires. The factors for other speeds can be calculated, since the product of the speed and factor yields a constant. Therefore $F = K/S$, where F = Factor, S = Revolutions per minute and K = Constant. This relationship was checked on standard oils. Each spindle of the Brookfield viscosimeter also yields a constant when the speed of rotation is multiplied by the factors furnished by the manufacturer.

When the MacMichael was used for determining the viscosity of dairy products, the use of different wires did not give equal viscosity values. However, a general trend prevailed in which the viscosity value decreased with an increase in the gauge of the wire.

In the standardization of the Mojonnier viscosimeter, the viscosity of the oils had an effect on the factors. The greater the viscosity, the larger were the factors for each plunger. This increase was the greatest for the small plunger, whereas the largest plunger showed only a slight difference with the various oils. The large and medium Mojonnier plungers gave viscosity values on dairy products which agreed fairly well with the values obtained with the other viscosimeters. However, the smallest plunger gave considerably lower values.

This study was not concerned with temperature effects but some mention should be made of them, since the Brookfield viscosimeter instructions make no statement regarding temperature. Standard oils at a temperature of 20 and 25° C. were used with the Brookfield and MacMichael viscosimeters. The temperature did not seem to change the factors furnished for the Brookfield viscosimeter but a decrease in temperature increased the factors for the MacMichael.

SUMMARY

Torsion pendulum viscosimeters operated under similar conditions gave satisfactory results in the determination of relative viscosity in dairy products. When tests in a series were to be compared, it was found advisable to use the same standardized instrument for all the tests and to maintain constant conditions with regard to speed of rotation, wire and plunger size, depth of immersion of plunger, temperature, and agitation, stirring or preparation of the sample.

Conversion factors were determined for the MacMichael and Mojonnier viscosimeters at 86° F. The factors furnished by the manufacturers of the Brookfield viscosimeter were found satisfactory. A decrease in the wire size and an increase in plunger size or in speed of rotation when applied to a given instrument caused a decrease in the apparent viscosity of various dairy products.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of C. F. Hufnagel during several phases of this work.

REFERENCES

- (1) HERSCHEL, W. H. The MacMichael Torsional Viscosimeter. *J. Ind. Eng. Chem.*, **12**, 3: 282-286. 1920.
- (2) HERSCHEL, W. H. The Improved MacMichael Viscosimeter. *J. Optical Soc. Am.* **7**, 4: 335-353. 1923.
- (3) SHEELY, M. L. The Standardization of Commercial Viscometers. *J. Ind. Eng. Chem.*, **15**, 11: 1109-1114. 1923.
- (4) WHITTAKER, R., AND HERRINGTON, B. L. The Calculation of Absolute Viscosity from Data Secured by Means of the Torsion Pendulum Viscosimeter. *J. Dairy Sci.*, **12**: 456-463. 1929.

PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. IV. EFFECT OF FORM OF VITAMIN A AND OF TOCOPHEROL SUPPLEMENTS ON CONCENTRATIONS OF VITAMIN A AND CAROTENOIDS¹

D. B. PARRISH, G. H. WISE,² AND J. S. HUGHES

Kansas Agricultural Experiment Station, Manhattan, Kansas

Previous studies (2, 10, 13) have demonstrated that giving massive amounts of vitamin A to dairy cows during the terminal weeks of gestation usually increases the vitamin A content of colostrum and early milk. Information was lacking, however, on the effects of dietary supplementation of tocopherols (vitamin E) and of different forms of vitamin A, alcoholic and esterified, on the concentrations of vitamin A and carotenoids in the early postpartum mammary secretions. Accordingly, a study of this problem was undertaken.

EXPERIMENTAL PROCEDURE

Vitamin A and carotenoid concentrations in colostrum and early milk from cows and heifers that received barn rations supplemented with vitamin A ester were compared with concentrations in similar secretions from cows that received the same rations supplemented with either vitamin A alcohol or vitamin A alcohol plus tocopherols in the free, unesterified form. This last combination of supplements was used because it was believed that any evidence of the sparing action of tocopherols would be manifested more clearly in conjunction with the less stable alcoholic form (11) than with the natural ester. Also, the vitamin A and carotenoid contents of the early mammary secretions from several cows receiving barn rations supplemented with tocopherols were compared with those from cows fed unsupplemented barn rations. Since no information on prepartal tocopherol supplementation was available, different levels were given.

Preparturient dairy cows that calved during a period from November, 1946, to February, 1947, were assigned on the basis of breed, number of lactations (first or later) and type and/or level of supplement fed to two major dietary groups, each consisting of three subgroups (table 1). Unfortunately, the limited number of animals available and three unavoidable casualties disrupted the equalization of subgroups. General feeding and management practices have been published (8).³ Types of vitamins, levels of administration and periods of supplementation are shown in table 1. All supplementation was discontinued at parturition. Variations in lengths of time cows received the

Received for publication January 11, 1949.

¹ Contribution no. 380, Department of Chemistry and no. 182, Department of Dairy Husbandry.

² Present address, Department of Animal Industry, North Carolina State College, Raleigh, N. C.

³ The identification of the groups is not the same as in the previous report and data on colostrum from additional cows are included in this study.

TABLE 1

Schedule of vitamin A and tocopherol supplementation of prepartal ration of dairy cows

Group	No. of cows	Days previous to date of expected parturition	
		28 - 14	13 - 0
<i>Ration supplement given</i>			
I a	7	500,000 I.U. vit. A ester	1,000,000 I.U. vit. A ester
b	8	500,000 I.U. vit. A alc.	1,000,000 I.U. vit. A alc.
c	6	500,000 I.U. vit. A alc. plus 0.5 g. α - γ tocopherols	1,000,000 I.U. vit. A alc. plus 1 g. α - γ tocopherols
II a	5	None	None
b	1	0.5 g. α - γ tocopherols	1 g. α - γ tocopherols
c 1	1	4 g. mixed tocopherols	4 g. mixed tocopherols
c 2	1	5 g. α - γ tocopherols	5 g. α - γ tocopherols
c 3	3	10 g. mixed tocopherols	10 g. mixed tocopherols

Note: α - γ tocopherols contained either 50 or 90% γ -tocopherol; all tocopherols were in the free, unesterified form.

supplements resulted from differences between expected and actual calving dates, but in no instance was the average time for each group less than that indicated in table 1.

Samples were collected from each milking during the first 4 days and on the eighth day postpartum. Vitamin A and carotenoids were determined on individual samples from the first four milkings only and on daily composites thereafter. Collection procedures and analytical methods have been reported (10).

RESULTS

Effect of form of vitamin A supplement. Group means (I-a and I-b, table 2) indicate that in the early stages of the transition from colostrum to milk the vitamin A content per unit of fat was greater in secretions from cows receiving the alcoholic form of vitamin A than from those receiving the esterified. Similar results were obtained when averages were computed on a per-unit-of-secretion basis (table 3). However, comparisons of data from paired animals of the two groups revealed that in several instances the higher values were in

TABLE 2

Vitamin A and carotenoid contents of fat in colostrum and early milk from groups of cows given different vitamin supplements

Group	Ration supplement	Number of milking						
		1	2	3	4	5 + 6a	7 + 8	15 + 16
Vitamin A (μg./g. of fat)								
I-a	Vit. A ester	113	96	71	46	33	23	9
I-b	Vit. A alcohol	139	145	93	64	36	21	9
I-c	Vit. A alc. and tocopherols	107	91	91	71	35	22	9
Carotenoids (μg./g. of fat)								
I-a	Vit. A ester	39	32	23	18	14	8	4
I-b	Vit. A alcohol	39	37	22	16	11	6	3
I-c	Vit. A alc. and tocopherols	36	31	23	18	11	7	4

* Composite samples.

TABLE 3

Vitamin A and carotenoid contents of colostrum and of early milk from groups of cows given different vitamin supplements

Ration Group supple- ment	Cows		Vitamin A					Carotenoids									
	Herd no. ^a	Lacta- tion	Number of milking					Number of milking									
			1	2	3	4	5 + 6 ^b	7 + 8	15 + 16	1	2	3	4	5 + 6 ^b	7 + 8	15 + 16	
<i>($\mu\text{g./100 ml. of secretion}$)</i>																	
I-a Vitamin A ester	H-132	1	740.	594	282	175	170	114	27	113	77	38	31	31	15	4	
	H-128	2	978	414	226	109	67	51	47	109	52	28	18	9	6	8	
	A-241	2	622	310	320	151	91	105	33	217	124	84	45	31	27	14	
	J-378	1	172	400	358	375	188	116	55	62	141	125	171	67	49	14	
	G-468	1	773	1,030	1,070	181	174	184	86	198	250	275	66	70	77	35	
	J-358	2	405	350	279	365	184	107	45	237	211	161	210	111	53	27	
	G-453	2	324	600	359	138	91	53	28	285	427	254	96	83	46	33	
	Mean		573	528	413	213	138	104	46	174	183	138	91	57	39	19	
<i>($\mu\text{g./100 ml. of secretion}$)</i>																	
I-b Vitamin A alcohol	H-137	1	990	646	292	214	119	95	35	135	88	30	17	17	8	0	
	A-252	1	1,185	1,420	972	575	98	125	77	143	214	105	64	11	11	18	
	H-115	3	460	320	169	195	113	62	47	140	92	45	51	33	16	12	
	A-243	2	450	1,050	970	514	301	112	57	70	130	122	72	50	29	12	
	J-379	1	287	439	248	350	229	140	17	83	146	68	96	75	48	4	
	G-465	1	870	660	520	226	149	121	39	478	175	253	99	66	63	27	
	J-365	2	355	776	338	176	110	116	43	116	230	91	46	38	37	23	
	G-452	2	620	685	407	236	142	62	34	388	383	235	157	102	36	30	
Mean		735	750	490	311	158	104	44	194	182	119	75	49	31	16		
<i>($\mu\text{g./100 ml. of secretion}$)</i>																	
I-c Vitamin A alcohol and toco- pherols	H-135	1	320	386	600	499	147	115	43	70	88	94	103	38	34	11	
	H-136	1	681	695	296	636	94	96	42	116	117	37	93	29	17	10	
	J-374	1	770	520	337	272	172	390	42	366	257	99	76	46	112	25	
	J-380	1	221	212	915	383	345	89	47	49	75	209	101	88	27	14	
	G-458	2	1,200	470	610	460	151	134	57	369	134	151	105	54	38	29	
	G-459	2	293	248	205	108	70	65	40	330	208	152	83	53	50	38	
Mean		581	422	494	393	163	148	45	217	147	124	94	51	48	21		

^aThe symbols H, A, J and G refer to the breeds Holstein, Ayrshire, Jersey and Guernsey, respectively.

^bComposite samples.

TABLE 4

Vitamin A and carotenoid contents of cholostrum and of early milk from groups of cows receiving either unsupplemented barn rations or the same rations plus tocopherol

Group	Ration supplement	Cows		Vitamin A							Carotenoids						
		Herd no. ^a	Lactation	Number of milking							Number of milking						
				1	2	3	4	5+6 ^b	7+8	15+16	1	2	3	4	5+6 ^b	7+8	15+16
		(μg./100 ml. of secretion)															
II-a	None	H-175	6	144	119	107	75	76	52	31	103	118	71	60	55	38	22
		H-105	4	446	262	119	77	72	48	38	156	112	55	40	30	21	20
		A-230	3	291	278	119	79	37	25	25	204	215	100	71	71	21	19
		G-432	5	213	135	81	67	54	40	27	368	250	121	95	75	54	34
		G-433	5	216	113	125	87	69	39	25	365	222	236	184	120	70	40
		Mean		262	181	110	77	70	43	29	239	183	117	95	70	41	27
II-b	0.5-1 g. toco-pherols	G-451	2	199	177	89	59	43	48	22	570	515	264	176	108	87	53
II-c ₁	4 g. toco-pherols	G-467	1	289	693	284	180	105	56	42	226	487	239	152	92	63	56
		A-240	2	215	182	125	36	68	33	8	138	117	86	23	41	23	27
II-c ₂	5 g. toco-pherols	H-138	1	582	852	112	100	51	35	22	133	192	33	36	22	12	7
		A-200	7	408	220	176	172	101	65	61	239	116	98	98	51	32	12
		J-365	2	316	227	155	113	104	74	43	359	248	176	127	120	73	36
		Mean, group II-c		362	435	170	120	86	53	35	219	232	126	87	65	41	28

^a The symbols H, A, J and G refer to the breeds Holstein, Ayrshire, Jersey and Guernsey, respectively.

^b Composite sample.

colostrum from cows fed vitamin A ester. Moreover, a pronounced individual variation among cows was observed. Thus, the superiority of one form of dietary vitamin A over the other for augmenting the levels of vitamin A in colostrum was not indicated.

The carotenoid contents of colostrum and early milk from cows receiving the alcoholic form of vitamin A also were similar to those from cows receiving the natural ester (tables 2 and 3).

Effect of supplements of tocopherols. Average levels of vitamin A in the first two samples of mammary secretions collected from cows receiving supplements of alcoholic vitamin A were higher than those from cows that also received tocopherols (tables 2 and 3). After the first two milkings, consistent differences were not apparent. In view of the wide variability among samples (table 3), the differences in the vitamin A contents of the mammary secretions from the two groups probably have little, if any, significance.

Although high levels of tocopherol supplementation of the ration during the later stages of gestation appeared to effect an increase in the average concentrations of vitamin A in colostrum and early milk (groups II-a and II-c, table 4), variations in results on samples from individual cows tend to nullify differences between means. Previous work (10) has shown that vitamin A levels generally are higher in colostrum from cows in their first lactation than in a later. Therefore, if comparisons of vitamin A levels are made only on colostrum from cows in their second or later lactations, the results from cows receiving tocopherols approach those from cows fed unsupplemented rations. Thus, as judged by these few data, it is questionable whether high levels of prepartal tocopherol supplementation substantially increase the amounts of vitamin A in colostrum and early milk.

The effect of feeding tocopherol supplements in conjunction with either normal dietary sources of vitamin A or massive amounts of vitamin A alcohol on carotenoid contents of the early mammary secretions were variable, and no consistent trends were discernible (tables 2, 3 and 4).

DISCUSSION

Previous work (9) has shown that the levels of vitamin A and its state of occurrence were practically the same in the blood of cows given supplements of either esterified or alcoholic vitamin A. Therefore, differences in the form of vitamin A ingested would not be expected to affect the quantities of this vitamin eliminated in the mammary secretions. Results of the present study tend to substantiate the foregoing view. Recent studies of vitamin A blood levels of Holstein heifers (12) also indicate that cattle utilize vitamin A alcohol and vitamin A ester to approximately the same degree.

Several reports cited by Hickman and Harris (6) have indicated a synergism between tocopherols and vitamin A in laboratory animals, but in another investigation (1) this relationship was not observed. Likewise, studies with cattle have not revealed this synergism when levels of vitamin A in either milk (5) or milk fat (14) were used as criteria. With the possible exception

of results on colostrum samples from cows given 10 g. of tocopherols daily, data reported herein are in accord with those from the foregoing milk investigations. Since it has been indicated that tocopherols are effective in increasing liver storage of vitamin A only when given at proper levels (6), the possibility that the cows might not have received proper levels and ratios of vitamins to disclose a synergism whereby tocopherols affect the levels of vitamin A in colostrum and early milk should not be overlooked.

Tocopherol supplements given to cows in post-colostral stages of lactation were reported to have increased concentrations of fat in the milk by 27 per cent (5), but in other trials the increases were not observed (3, 4, 14). The present study did not reveal any definite effect of tocopherol supplementation on either fat levels in or yields of colostrum. However, it should be noted that the fat content of early colostric secretions may vary widely among different cows and from milking to milking from the same cow (7). Thus, unless the effects of tocopherol supplementation are more marked than the normal variations, differences would not be detectable.

SUMMARY

The rations of dairy cows and heifers were supplemented with either various amounts of tocopherols or with large quantities of vitamin A ester, vitamin A alcohol or vitamin A alcohol plus tocopherols during the terminal 4 weeks (average minimum) of gestation. The relative effects of these prepartal supplements on vitamin A and carotenoid concentrations of colostrum and early milk were investigated.

In view of the individual variation observed within the same groups, differences found in vitamin A concentrations in colostrum and early milk could not be ascribed to the form or combination of vitamin supplements given. Thus, vitamin A ester, vitamin A alcohol and vitamin A alcohol plus tocopherols appeared to be of a similar value in affecting increases in vitamin A levels of colostrum and early milk.

In a trial with a limited number of cows, addition of tocopherols at various levels to barn rations did not increase substantially vitamin A content of colostrum and early milk.

Neither the form of vitamin A supplement given nor the addition of tocopherols had a significant effect on carotenoid levels of the early mammary secretions.

ACKNOWLEDGMENT

The authors wish to thank Distillation Products, Inc., Rochester, N. Y., for providing tocopherols and vitamin A ester, and the Borden Co., New York, N. Y., for furnishing vitamin A alcohol and vitamin A ester used in this study. William Mudge, Grant Moody and R. J. Flipse assisted with various phases of the study.

REFERENCES

- (1) DUBOULOZ, P., AND GASQUY, C. Action de l' Esterification et du Tocophérol sur le Mise en Réserve de la Vitamine A chez le Rat. *Compt. rend. soc. biol.*, 140: 621-622. 1946.

- (2) ESH, G. C., SUTTON, T. S., HIBBS, J. W., AND KRAUSS, W. E. The Effect of Soya-Phosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, 31: 461-478. 1948.
- (3) FOUNTAINE, F. C., AND PARRISH, D. B. Unpublished data, Kansas Agricultural Experiment Station. 1948.
- (4) GULLICKSON, T. W., FITCH, J. B., AND GILMORE, L. O. Effect of Feeding Tocopherols to Dairy Cows on the Quantity and Fat Content of Milk Produced. *J. Dairy Sci.*, 31: 557-560. 1948.
- (5) HARRIS, P. L., SWANSON, W. J., AND HICKMAN, K. C. D. Covitamin Studies. VI. Effect of Tocopherol Supplementation on the Output of Vitamin A, Carotene and Fat by Dairy Cows. *J. Nutrition*, 33: 411-428. 1947.
- (6) HICKMAN, K. C. D., AND HARRIS, P. L. Tocopherol Interrelationships. *Advances in Enzymol.*, 6: 469-524. 1946.
- (7) PARRISH, D. B., WISE, G. H., ATKESON, F. W., AND HUGHES, J. S. Properties of the Colostrum of the Dairy Cow. V. Variations in Yields and in Concentrations of Selected Constituents During the Transition Period. Unpublished data, Kansas Agricultural Experiment Station. 1946.
- (8) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S. Properties of the Colostrum of the Dairy Cow. I. Tocopherol Levels in the Colostrum and in the Early Milk. *J. Dairy Sci.*, 30: 849-860. 1947.
- (9) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S. Effect of Vitamin A Supplements Upon the State of Vitamin A in Blood Serum of the Dairy Cow and in Blood Serum and Liver of Its Neonatal Calf. *J. Biol. Chem.*, 172: 355-365. 1948.
- (10) PARRISH, D. B., WISE, G. H., ATKESON, F. W., AND HUGHES, J. S. Properties of the Colostrum of the Dairy Cow. III. Several Factors Affecting Vitamin A and Carotenoid Content. *J. Dairy Sci.*, 32: 209-221. 1949.
- (11) ROSENBERG, H. R. *Chemistry and Physiology of the Vitamins*. 1st Ed. Interscience Publishers, Inc. 1942.
- (12) ROSS, R. H., KNOTT, C. B., AND GUERRANT, N. B. The Utilization of β -Carotene, Vitamin A Alcohol, and the Natural Ester of Vitamin A by Holstein Heifers. *J. Dairy Sci.*, 31: 111-117. 1948.
- (13) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., WHITING, F., NORTON, C. L., AND TURK, K. L. The Relationship of the Prepartum Diet to the Carotene and Vitamin A Content of Bovine Colostrum. *J. Dairy Sci.*, 30: 343-350. 1947.
- (14) WHITING, F., LOOSLI, J. K., KRUKOVSKY, V. N., AND TURK, K. L. The Influence of Tocopherols and Cod-Liver Oil on Milk and Fat Production. *J. Dairy Sci.*, 32: 133-138. 1949.

THE NUTRITIVE VALUE OF ALFALFA HAY. III. CORN AS A SUPPLEMENT TO AN ALL-ALFALFA HAY RATION FOR MILK PRODUCTION¹

C. F. HUFFMAN AND C. W. DUNCAN

*The Departments of Dairy and Agricultural Chemistry,
Michigan Agricultural Experiment Station, East Lansing*

In previous reports from this Station (13, 14, 15, 16), it has been demonstrated that milk production can be increased when properly depleted cows have had a part of the alfalfa hay in the ration replaced by concentrates on an equal total digestible nutrient basis. Several other investigators also have reported that cows fed alfalfa hay alone do not utilize efficiently the total digestible nutrients for milk production except in the early stages of lactation (8, 9, 11, 12, 19, 22, 23). All of these results suggest the possibility that the decreased amount of milk produced by feeding alfalfa hay alone may be due to certain dietary deficiencies.

Since alfalfa hay is sometimes low in phosphorus, Haag *et al.* (8, 10) determined the phosphorus balances of cows on an all-alfalfa hay ration and found that the balances always were negative. Huffman and Duncan (17) have shown further that the addition of a phosphorus supplement in the form of bone meal to an all-alfalfa hay ration failed to prevent the decline in milk production.

Haag (9) and Wright and Haag (27) showed that L-cystine had a favorable supplementary effect upon rats when alfalfa leaf meal was fed at a 9 per cent protein level. On the other hand, Huffman and Duncan (14) reported that the ingestion of 20 g. of L-cystine per day per cow as a supplement to an all-alfalfa hay ration produced no significant effect on milk production.

Kellner and Köhler (20) found that the addition of 1 kg. of digestible protein starch, cane sugar, crude fiber or fat per day to a maintenance ration of steers resulted in the production of 235, 248, 188, 253 and 474-598 g., respectively, of fat. Their experiments with concentrates and roughages also showed that the total digestible nutrients in roughages produced less than the calculated amount of fat. As a result of their work, the production of fat from digestible nutrients would appear to vary with the crude fiber content of the ration. The higher the fiber content, the less the feeding value of the digestible nutrients. These results provide the basis of the present discount systems, such as "starch values", "net energy values" and "productive energy values", used in the evaluation of feedstuffs. Huffman and Duncan (15), using properly depleted cows, have shown that the addition of either corn starch or corn sugar to an all-alfalfa hay ration did not cause an increase in milk production. When 6 lb. of corn replaced 6 lb. of starch or glucose, however, milk production always increased. The level of crude fiber in the rations remained unchanged in both the corn-feeding and starch- or sugar-feeding periods, which suggests that

Received for publication January 17, 1949.

¹ Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1015 (n.s.).

the corn grain contributed a dietary factor or factors needed for milk production which are not present in the alfalfa hay or in the corn starch or corn sugar. These experiments also eliminated a lack of available energy as the factor responsible for the low producing power of alfalfa hay.

Smith *et al.* (24) studied the effect on milk production by replacing 13 to 25 per cent of the total digestible nutrients in an all-alfalfa hay ration, on an equal total digestible nutrient basis, with various concentrates. Soybeans were most effective in increasing milk production, whereas cane molasses and sugar were least effective. Davis and Kemmerer (1) reported that milk production was increased when dried grapefruit peel was added to an all-alfalfa hay ration.

The investigation reported in this paper was made for the express purpose of obtaining additional information on the amount of milk produced when a part of the digestible nutrients in alfalfa hay was replaced with corn.

EXPERIMENTAL

Seven Holstein cows (A5, A18, D5, D12, D14, 266 and 267), three Jersey cows (74, 77 and 78), and two Brown-Swiss cows (A45 and 238) were used in the 15 trials reported in this paper. Cows A5, A18 and D12 were used on two different trials but with different hays.

The depletion technic developed at this Station to exhaust the cows of their reserve milk-producing factors consists of the following practice: The cows are placed on an all-alfalfa hay ration (a) either at the time of parturition or (b) at an advanced stage of lactation and are continued on this regime until they are depleted of the factor(s) needed to balance the total digestible nutrients in the hay. The depletion period usually required from 6 to 8 weeks for almost all of the cows used in this study; however, the cows in a more advanced stage of lactation usually were depleted within 2 weeks. Depletion of the factor(s) was indicated by an initial decline and then a leveling-off in milk production. Both types of depleted cows were used in this study. The period just prior to the replacement of a part of the total digestible nutrients with corn was used as the basal period—usually a 15-day period. The cows were fed twice a day and had water available in drinking cups at all times. They were weighed at the same hour every third day. The cows were milked twice a day and the milk was weighed after each milking. The equivalent number of pounds of 4 per cent fat-corrected milk was calculated by the formula proposed by Gaines (4). Three-day composite samples were taken for butterfat determinations.

The 11 alfalfa hays used in this investigation represented 8 crop years. Most of these hays were, or would have been, graded U. S. grade no. 2 as to color and leafiness, second cutting, and harvested at about the half-bloom stage. The hay fed to A45 was a mixture of alfalfa and alsike clover. The digestible protein and total digestible nutrients of all of the hays were determined by digestion experiments by using either yearling heifers, dry cows or cows in milk, except those hays used in trials 1, 3, 8 and 15. Ten-day collection periods were used for the determination of these data. The animals were fed the hays under consideration for from 10 days to several months prior to the collection periods. The digestible

TABLE 1
Description of the hays, their chemical composition and their actual or
calculated coefficients of digestibility

Trial no.	Cow no.	Moisture	Ash	Protein	Ether ext.	Crude fiber	N.F.E.	Dig. proteins	T.D.N.	Description of the hays
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
1*	D12	12.6	7.19	15.8	2.18	25.4	36.8	11.7	50.7	U.S. No. 2, 2nd cut. alf., 1936 crop
2	D12	13.1	6.65	15.0	1.80	27.2	36.3	10.2	49.7	U.S. No. 1, 1st cut. bud alf., 1937 crop
3	D14	13.8	7.82	16.4	1.80	28.3	31.9	11.8	48.0	U.S. No. 1, 2nd cut. alf., 1937 crop
4	74 ^c	11.9	6.88	15.0	2.77	28.6	34.9	10.2	47.2	U.S. No. 2, 2nd cut. alf., 1938 crop
5	266			68	28	39	69			
6	78									
7	239	14.2	5.94	16.6	2.18	31.0	30.1	11.5	46.1	Ungraded, 2nd cut. alf., 1938 crop
8	D5	10.7	6.31	14.9	2.33	27.0	38.8	11.0	52.3	U.S. No. 3, 1st cut. alf., 1939 crop
9	A18 ^d	10.9	6.76	15.9	2.59	28.1	35.8	11.8	51.7	Ungraded, 2nd cut. alf., 1941 crop
10	A5			74	32	45	71			
11	77	11.8	6.30	16.5	1.67	29.9	33.8	11.1	48.3	U.S. No. 1, 1st cut. bud alf., 1941 crop
12	A5	15.4	5.89	13.1	1.72	28.1	35.8	8.8	49.3	Ungraded, 2nd cut. alf., 1942 crop
13	A18 ^d	13.0	6.43	14.6	1.74	28.6	35.6	10.5	49.7	Ungraded, 2nd cut. alf., 1943 crop
14	267			72	39	47	68			
15	A45	10.5	5.99	14.2	2.01	30.4	36.9	10.2	51.0	Ungraded, 1st cut. alf.-alsike, 1944 crop
				72 ^b	34	43	71			

* The first line in each trial represents the chemical composition of the hay.

^b The second line in each trial represents the coefficients of digestibility of the various hay fractions. Those marked with footnote^b represent the calculated values, whereas all other values were obtained experimentally.

^c Three cows (74, 266 and 78) were fed this hay.

^d Two cows each in trials 9 and 10 and 13 and 14 were fed the same hay.

protein and total digestible nutrient contents of the other 4 hays were calculated from the chemical analyses and by the use of the coefficients of digestibility recommended by Morrison (21). The hay data are presented in table 1.

TABLE 2
The chemical composition, digestible protein and total digestible nutrient content
of the corn used in each trial

Trial no.	Cow no.	Moisture	Ash	Protein	Ether ext.	Crude fiber	N.F.E.	Dig. protein	T.D.N.
		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	D12	17.2	2.71	9.94	4.09	2.00	64.1	7.7	76.7
2	D12	12.9	2.97	9.19	3.49	2.20	69.3	7.1	79.8
3	D14	12.7	2.05	10.13	4.23	1.93	69.0	7.8	81.7
4	74	13.6	4.73	8.62	3.92	2.93	66.5	6.6	77.9
5	266	12.6	3.10	9.44	4.00	3.02	67.8	7.3	80.2
6	78	12.6	3.10	9.44	4.00	3.02	67.8	7.3	80.2
7	239	13.7	2.43	9.13	2.32	3.19	69.2	7.0	78.0
8	D5	13.1	1.77	8.93	3.58	2.88	69.7	6.9	80.6
9	A18	12.5	1.92	8.94	4.36	3.01	69.3	6.9	81.8
10	A5	12.5	1.92	8.94	4.36	3.01	69.3	6.9	81.8
11	77	12.5	1.92	8.94	4.36	3.01	69.3	6.9	81.8
12	A5	14.2	2.22	9.06	3.95	2.57	68.0	7.0	79.6
13	A18	12.2	2.44	8.33	4.22	2.72	70.1	6.4	81.7
14	267	12.2	2.44	8.33	4.22	2.72	70.1	6.4	81.7
15	A45	13.4	2.65	7.88	3.86	3.01	69.2	6.1	80.0

Number 2 yellow dent corn was ground to medium fineness and used in all of the trials. The digestible protein and total digestible nutrient contents of the various batches of corn were calculated from actual chemical analyses and the use of the coefficients of digestibility recommended by Morrison (21). The corn data are presented in table 2.

Short-time experimental periods, usually 15-day periods, were used in order to minimize the effect of the natural tendency of cows to decline in milk with the

TABLE 3

The data pertaining to the stage of lactation, body weights, the average daily yield of 4 per cent fat-corrected milk, alfalfa hay and corn intakes and the total digestible nutrients received and required

Trial no.	Cow no.	Exptl. period	In milk	Body wt.	F.C.M.		Feed intake of:			T.D.N.	
					Yield	Incr.	Hay	Corn	D.P. ^a	Rec.	Req.
		(days)	(days)	(lb.)	(lb.)	(%)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
1 ^b	D12	15	120	1191	23.0		44.6		5.22	22.6	16.6
		33	135	1225	31.2	35.7	30.0	9.0	4.20	22.1	19.4
2	D12	15	179	1260	25.2		45.0		4.59	22.4	17.8
		12	194	1273	29.0	15.1	30.0	9.0	3.70	22.1	19.1
3	D14	9	110	1245	23.3		44.2		5.22	21.2	17.1
		15	119	1233	26.1	12.0	29.7	9.0	4.20	21.6	17.8
4	74	15	324	770	11.3		27.4		2.79	12.9	9.9
		9	339	763	12.6	11.5	11.3	9.0	1.74	12.3	10.2
5	266	15	152	1020	16.0		35.6		3.63	16.8	13.2
		15	167	999	19.4	21.3	20.0	9.0	2.70	16.6	14.1
6	78	15	261	890	8.4		24.2		2.47	11.4	9.8
		15	276	845	11.0	31.0	10.0	9.0	1.68	11.9	10.4
7	239	9	99	1143	23.4		41.2		4.74	19.0	16.4
		18	108	1116	26.8	14.5	27.0	9.0	3.74	19.4	17.3
8	D5	12	155	1067	12.1		34.4		3.78	18.0	12.2
		15	167	1065	14.0	15.7	20.0	7.5	2.72	16.5	12.8
9	A18	12	262	1214	14.4		38.8		4.58	20.1	13.9
		15	274	1189	18.3	27.1	24.9	9.0	3.56	20.2	15.1
10	A5	12	183	1155	15.6		34.1		4.02	17.6	13.9
		15	195	1134	21.3	36.5	20.0	9.0	2.98	17.7	15.7
11	77	18	144	768	16.0		24.8		2.75	12.0	11.4
		15	162	741	17.4	8.8	15.0	6.0	2.08	12.1	11.7
12	A5	15	276	1193	16.0		39.7		3.49	19.6	14.3
		21	291	1196	18.9	18.1	25.0	9.0	2.83	19.5	15.3
13	A18	24	265	1187	11.8		34.5		3.62	17.1	13.0
		30	289	1207	12.7	7.6	20.0	9.0	2.68	17.3	13.3
14	267	15	228	1238	15.5		34.9		3.66	17.3	14.5
		24	243	1200	17.8	14.8	20.0	9.0	2.68	17.3	15.0
15	A45	12	266	1006	7.0		28.7		2.93	14.6	10.2
		15	278	1003	9.6	37.1	15.7	6.0	1.97	12.8	11.0

^a D.P. = digestible protein.

^b The first line in each trial represents the all-alfalfa hay ration, whereas the second line represents the alfalfa-corn ration.

advance in lactation. The length of the periods when the hay-corn ration was fed varied in a few cases from 8 to 32 days. The data showing the effect of replacing a part of the total digestible nutrients in hay with an equal amount of total digestible nutrients in the form of corn are presented in table 3. The immediate effect on milk production in properly depleted cows when they are changed from an all-alfalfa hay ration to a hay-corn ration and back to an all-alfalfa hay ration again is strikingly illustrated in figure 1. The average daily milk pro-

duction of 5 typical cows (A5, A18, D12, D14 and 266) was used in the preparation of this figure.

RESULTS

The data showing the stages of lactation, body weights, the average daily yields of 4 per cent fat-corrected milk, hay and corn consumption, the total digestible nutrients received and required and the digestible protein intake are presented in table 3. When corn replaced a part of the hay, there was a marked reduction in digestible protein intake. Nine pounds of corn replaced about 15 pounds of alfalfa hay in 12 trials. In the case of cow D12 (trial 8), 7.5 lb. of corn replaced 14.4 lb. of hay, whereas in trials 11 and 15, 6 lb. of corn replaced 9.8 and 13 lb. of hay, respectively.

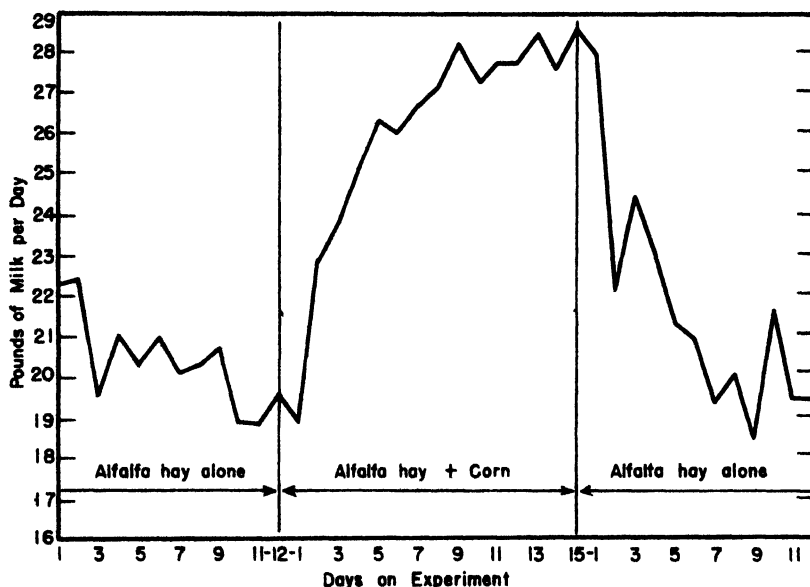


FIG. 1. The effect on milk production in properly depleted cows when changed from an all-alfalfa hay ration to an alfalfa-corn ration and back again to an all-alfalfa hay ration. (Av. for 5 cows)

The cows used in trials 5, 9, 10-14 consumed either the same amount or within plus or minus 0.2 lb. of total digestible nutrients per day during both the alfalfa hay and alfalfa-corn feeding periods. The cows used in trials 1, 2, 4, 8 and 15 consumed from 0.3 to 1.8 lb. less total digestible nutrients per day during the alfalfa-corn feeding periods than during the alfalfa hay periods. The cows used in trials 3, 6 and 7 consumed from 0.4 to 0.5 lb. more total digestible nutrients during the alfalfa-corn periods than during the all-alfalfa hay period. In all trials, however, the total digestible nutrient intake was greater than that required.

Insofar as possible, 15-day experimental periods were used in both the alfalfa-corn and all-alfalfa hay feeding periods. In the case of the cows used in trials 3, 7, 8, 9, 10 and 15, shorter periods were utilized because they had become depleted

sooner. On the other hand, cows 77 (trial 11) and A18 (trial 13) did not become entirely depleted in the usual length of time. Cow 74 (trial 4) only remained on the hay-corn ration for 9 days, since it became necessary to discontinue milking in preparation for the next calving. In the case of cow D12 (trial 2), the shorter hay-corn period was necessitated because of the limited amount of alfalfa hay cut in the bud stage. Cows D12 (trial 1), 239 (trial 7), A5 (trial 12), A18 (trial 13) and 267 (trial 14) were continued on the alfalfa-corn ration for more than 15 days.

The stage of lactation at the beginning of the alfalfa hay feeding period varied from 99 to 324 days. The mean body weights show there was a negligible loss of weight in 11 trials (maximum loss 45 lb., average 20.7 lb.) when the cows were changed from an all-alfalfa hay ration to an alfalfa-corn ration, and a gain in weight in 4 trials (maximum gain 34 lb., average 17.5 lb.). The gains or losses in weight do not appear to be significant in view of the relatively short periods employed and the reduction in dry matter intake during the alfalfa-corn feeding periods.

Since there were no significant differences in the per cent of fat in the milk between the all-alfalfa hay and alfalfa-corn feeding periods, only the average daily milk production records, on the 4 per cent fat-corrected basis, are presented. The increase in milk production varied from 0.9 to 8.2 lb. per day when part of the hay was replaced by corn on an equal total digestible nutrient basis. The increases varied from 7.6 to 37.1 per cent. In 6 trials, the increase was more than 20 per cent, whereas the increase was only 12 per cent or less in 4 trials. The wide differences in response to the change from the all-alfalfa hay ration to alfalfa and corn are attributed to variations in the hay, stage of lactation, stage of gestation, and inheritance for milk production.

DISCUSSION

The partial replacement of alfalfa hay with corn in the ration of properly depleted cows resulted in an increase in the production of 4 per cent fat-corrected milk. These results appear significant in view of the fact that 11 different hays were used, representing 8 different crop years. Although two of these hays were cut in the bud stage (trials 2 and 11), milk production increased above the all-alfalfa hay ration when corn replaced part of the hay. The greatest percentage increase in fat-corrected milk occurred in trial 15, where only 6 lb. of corn replaced 13 lb. of first cutting alfalfa-alsike clover hay. This cow had completed 278 days of lactation at the time of the change, yet she was able to produce 2.6 lb. more fat-corrected milk per day during the 15-day period than when on hay alone, or a 37.1 per cent increase. Cow 74 (trial 4) produced 1.3 lb. more fat-corrected milk per day after she was changed to an alfalfa-corn ration on the 339th day of lactation. The increased fat-corrected milk resulting from the partial replacement of the total digestible nutrients in hay with corn is in agreement with the results of Smith *et al.* (24), who reported an increase in milk production when a part of the total digestible nutrients of an all-alfalfa hay ration was replaced by either fish meal, meat meal, beet pulp, wheat bran, blood meal, pea meal or soybean meal.

The improvement in milk production which resulted from partial replacement of the total digestible nutrients of alfalfa hay with corn was not due to an increased intake of digestible protein since there was a marked reduction in digestible protein intake during each alfalfa-corn feeding period. The possibility of an amino acid deficiency appears unlikely in view of the earlier work which indicated that the ingestion of cystine as a supplement to an all-alfalfa hay ration failed to give a favorable response (14). Smith *et al.* (24) concluded that there were no indications that improved milk production resulted from improving the quality of protein in the ration.

The milk production obtained during the all-alfalfa hay feeding periods was not due to a lack of available energy, inasmuch as it previously has been shown that the addition of corn starch or corn sugar failed to increase milk production in properly depleted cows (15). These findings are supported by those of Smith *et al.* (24) who found that milk production always was less when sugar or molasses were added to the ration than with any other concentrates tested. The total digestible nutrient intake always was in excess of that required when the cows were on the all-alfalfa hay ration.

In this investigation the increased milk production which resulted from replacing a part of the hay with corn, on an equal total digestible nutrient basis, was associated with a reduction in the crude fiber intake. Kellner and Köhler (20) fed steers a maintenance ration and then superimposed various feeds on it to study changes in body weights. They concluded that the productive values of roughages varied with the crude fiber content. These investigators used the term "starch value" to denote the probable nutritive value of feeds by discounting the total digestible nutrients on the basis of their crude fiber content. In a previous publication (15), data have been presented which indicate that the level of crude fiber in the ration does not offer an adequate explanation of Kellner's hypothesis, because the replacement of 6 lb. of starch or glucose by 6 lb. of wheat or corn always resulted in an increase in milk production. These results serve to indicate that corn contains an unidentified factor(s) needed to balance the deficiencies present in an all-alfalfa hay ration. Additional evidence has been supplied by Graves *et al.* (5, 6) to show that the total digestible nutrient discount system, based entirely on crude fiber, does not apply to alfalfa hay. These investigators reported that cows produced 2.96 lb. of fat-corrected milk per lb. of total digestible nutrients intake on an all-alfalfa hay ration and 2.50 lb. of milk on an alfalfa hay-grain ration, but the cows on the hay-grain ration produced more pounds of milk per lactation period than those on alfalfa alone. The reduced efficiency of total digestible nutrient utilization by the cows on the hay-grain ration probably was due to the higher plane of nutrition. Graves (7), however, attributes the lower lactation records of the cows on alfalfa alone to their inability to consume sufficient total digestible nutrients, rather than to a deficiency in the hay. The alfalfa hays used in the above experiment were grown at Huntley, Montana, Woodward, Oklahoma, and Mandan, North Dakota, and contained 31.3, 29.2 and 31.5 per cent crude fiber, respectively. These hays contained more crude fiber than most of the hays fed in the present investigation.

Several investigators have pointed out that the total digestible nutrient content of a ration composed of alfalfa alone is not utilized efficiently for milk production except during the early stages of lactation (8, 9, 11, 12, 19, 22, 23). An increase in the digestibility of the ration when corn replaces a part of the hay is unlikely in view of the results obtained by Watson *et al.* (25) with steers and sheep. They found that the digestibility of barley was the same when it was fed in combination with timothy or alfalfa hay as when fed alone. Forbes *et al.* (2) fed steers alfalfa hay alone, corn alone, and alfalfa plus corn and concluded from this work that the digestibility of corn meal is not materially different when fed in combination with alfalfa or when fed alone. They also observed that the heat increment per pound of dry matter consumed was higher when alfalfa hay or corn was fed alone than when a combination of the two was fed. This is further evidence to indicate that the increased efficiency in milk production obtained when a part of the total digestible nutrients in an all-alfalfa hay ration is replaced with corn is due to an unidentified factor(s) in the corn, rather than due to an excess of crude fiber.

The possibility that the milk-stimulating effect produced by corn is a rumen phenomenon is suggested also by the work of Forbes *et al.* (2), who found that more methane was produced when a mixed ration of alfalfa and corn was fed than when either constituent was fed alone. Hunt *et al.* (18) reported that the addition of ground corn to the hay ration of steers resulted in an increased synthesis of riboflavin.

The immediate effect on the milk production of properly depleted cows when part of the alfalfa hay is replaced with corn, on an equal total digestible nutrient basis, is shown in figure 1. A marked increase in milk production occurs on the second day following the inclusion of corn in the ration and persists until the peak of production is reached in about 7 to 10 days. When corn is removed from the ration, a significant drop in milk production occurs on the second day and the downward trend continues for about a week or longer before production tends to level out.

SUMMARY

Twelve cows which had been depleted of their reserve milk-producing factor(s) on an all-alfalfa hay ration were used in 15 trials to study the effect on milk production after a part of the total digestible nutrients in alfalfa had been replaced with corn. Eleven different hays representing 8 crop years were used and each experimental period averaged 15 days in length.

The replacement of a part of the alfalfa hay with corn, on an equal total digestible nutrient basis, always resulted in an increased production of 4 per cent fat-corrected milk.

Milk production increased markedly during the second day following the change to the alfalfa-corn ration and persisted for 7 to 10 days before reaching a plateau. An equally sharp drop in milk production occurred on the second day following the change to an all-alfalfa hay ration and the drop continued for one week or longer before production stabilized at a lower level.

Possible explanations for the increased production are discussed.

The results of this investigation indicate that the corn grain supplied an unidentified factor(s) needed to balance alfalfa hay for milk production.

REFERENCES

- (1) DAVIS, R. N., AND KEMMERER, A. R. Lactating Factors for Dairy Cows in Dried Grapefruit Peel. *J. Dairy Sci.*, **31**: 973-975. 1948.
- (2) FORBES, E. B., BRAMAN, W. W., KRISS, M., SWIFT, R. W., MILLER, R. C., FRENCH, R. B., LETONOFF, T. V., AND SHARPLESS, G. R. The Metabolizable Energy and Net Energy Values of Corn Meal When Fed Exclusively and in Combination with Alfalfa Hay. *J. Agr. Research*, **43**: 1015-1026. 1931.
- (3) FRAPP, G. S. The Composition and Utilization of Texas Feeding Stuffs. *Tex. Agr. Expt. Sta. Bull.* 461 rev. 1947.
- (4) GAINES, W. L. The Energy Basis of Measuring Milk Yield in Dairy Cows. *Ill. Agr. Expt. Sta. Bull.* **308**. 1928.
- (5) GRAVES, R. R., DAWSON, J. R., KOPLAND, D. V., WATT, A. L., AND VAN HORN, A. G. Feeding Dairy Cows on Alfalfa Hay Alone. *U. S. Dept. Agr. Tech. Bull.* **610**. 1938.
- (6) GRAVES, R. R., DAWSON, J. R., AND KOPLAND, D. V. Relative Values for Milk Production of Hay and Silage Made from Immature Pasture Herbage. *U. S. Dept. Agr. Tech. Bull.* **649**. 1938.
- (7) GRAVES, R. R. Can Good Producing Cows be Fed in Such Manner as to Maintain Their Weight? *J. Dairy Sci.*, **30**: 41-47. 1947.
- (8) HAAG, J. R., JONES, J. S., JONES, I. R., AND BRANDT, P. M. The Physiological Effect of Rations Restricted Principally or Solely to the Alfalfa Plant. I. The Calcium, Phosphorus and Nitrogen Metabolism of Dairy Cattle. *J. Dairy Sci.*, **12**: 445-455. 1929.
- (9) HAAG, J. R. The Physiological Effect of Rations Restricted Principally or Solely to the Alfalfa Plant. II. Cystine as a Limiting Factor in the Nutritive Value of Alfalfa Proteins. *J. Nutrition*, **4**: 363-370. 1931.
- (10) HAAG, J. R., JONES, I. R., AND BRANDT, P. M. The Physiological Effect of Rations Restricted Principally or Solely to the Alfalfa Plant. III. The Influence of Various Mineral Supplements in the Calcium, Phosphorus and Nitrogen Metabolism of Dairy Cattle. *J. Dairy Sci.*, **15**: 23-28. 1932.
- (11) HART, E. B., HUMPHREY, G. C., AND MORRISON, F. B. Comparative Efficiency for Growth of the Nitrogen of Alfalfa Hay and Corn Grain. *Wis. Agr. Expt. Sta. Research Bull.* **33**. pp. 108-119. 1914.
- (12) HEADLEY, F. B. Feeding Experiments with Dairy Cows. *Nev. Agr. Expt. Sta. Bull.* **119**. 1930.
- (13) HUFFMAN, C. F. A Method of Studying the Deficiencies of Alfalfa Hay and the Feeding Value of Various Feeds as Supplements to Alfalfa Hay. *J. Dairy Sci.*, **21**: 101-102. 1938.
- (14) HUFFMAN, C. F., AND DUNCAN, C. W. The Nutritive Value of Alfalfa Hay. I. Cystine as a Supplement to an All-Alfalfa Hay Ration for Milk Production. *J. Dairy Sci.*, **25**: 507-515. 1942.
- (15) HUFFMAN, C. F., AND DUNCAN, C. W. The Nutritive Value of Alfalfa Hay. II. Starch and Glucose as Supplements to an All-Alfalfa Hay Ration. *J. Dairy Sci.*, **27**: 821-833. 1944.
- (16) HUFFMAN, C. F., AND DUNCAN, C. W. Unknown Dietary Factor or Factors Needed by Lactating Cows Depleted on Legume Hay Alone. *Federation Proc.*, **6**, II: 409-410. 1947.
- (17) HUFFMAN, C. F., AND DUNCAN, C. W. Unpublished data. *Mich. Agr. Expt. Sta.* 1947.
- (18) HUNT, C. H., BURROUGHS, E. W., BETHEKE, R. M., SCHALK, A. F., AND GERLAUGH, P. Further Studies on Riboflavin and Thiamine in the Rumen Content of Cattle. II. *J. Nutrition*, **25**: 207-216. 1943.

- (19) JONES, I. R., BRANDT, P. M., AND HAAG, J. R. Studies with Alfalfa Hay for Milk Production. *Oreg. Agr. Expt. Sta. Bull.* 328. 1934.
- (20) KELLNER, T., AND KÖHLER, A. Untersuchungen über den Stoff- und Energie-Umsatz des erwachsenen Rindes bei Erhaltungs- und Produktionsfutter. *Landw. Vers. Sta.*, 53: 1-474. 1900.
- (21) MORRISON, F. B. Feeds and Feeding. 21st Ed. The Morrison Publishing Co., Ithaca, N. Y. 1948.
- (22) REED, O. E., FITCH, J. B., AND CAVE, H. W. The Relation of Feeding and Age of Calving to the Development of Dairy Heifers. *Kans. Agr. Expt. Sta. Bull.* 233. 1924.
- (23) SHERWOOD, D. H., AND DEAN, H. K. Feeding Alfalfa Hay Alone and with Concentrates to Dairy Cows. *Oreg. Agr. Expt. Sta. Bull.* 380. 1940.
- (24) SMITH, V. R., JONES, I. R., AND HAAG, J. R. Alfalfa with and without Concentrates for Milk Production. *J. Dairy Sci.*, 28: 343-354. 1945.
- (25) WATSON, C. J., KENNEDY, J. W., DAVIDSON, W. M., ROBINSON, C. H., AND MUIR, G. W. Digestibility Studies with Ruminants. X. Relative Associative Effects of the Roughages, Timothy and Alfalfa, with Barley. *Sci. Agr.*, 27: 175-182. 1947.
- (26) WOLL, F. W. Alfalfa as a Sole Feed for Dairy Cattle. *J. Dairy Sci.*, 1: 447-461. 1918.
- (27) WRIGHT, L. D., AND HAAG, J. R. The Lactation-Promoting Effect of L-Cystine when Fed with Alfalfa Proteins. *J. Nutrition*, 17: 263-268. 1939.

THE YEAST IN THE SURFACE SMEAR OF BRICK CHEESE¹

K. K. IYA² AND W. C. FRAZIER

*Department of Agricultural Bacteriology
University of Wisconsin, Madison, Wisconsin*

In the manufacture of brick cheese, the appearance of a slimy surface smear, slightly orange in color, is a prerequisite for desirable qualities in the finished cheese. This surface smear has been investigated by several workers. Langhus *et al.* (1) showed that the smear contained micrococci, *Bacterium linens* and some "yeast-like" organisms. It was felt that a study of the yeast and its probable role in the ripening of brick cheese might yield interesting results.

The samples of brick cheese either were prepared in the Department of Dairy Industry at this University or were obtained from factories located in the vicinity of Madison. Contact smears of the surface of the cheeses were made on successive days after the salting of the cheese, and the microbial types were examined. The cocci developed first within 3 days. Between the third and the eighth day after salting of the cheese, the yeasts made their appearance, increased in numbers and gradually disappeared. After about the eighth day, microscopic examination revealed large numbers of gram-positive rods which were identified as *B. linens*. In a few days the smears showed practically only *B. linens*.

The yeast was isolated in pure culture and, as a result of a study of its various characters, was identified as a *Mycoderma* species of the family *Cryptococcaceae*, according to the system of Lodder as described by Skinner *et al.* (2).

The yeast forms a dry wrinkled pellicle on liquid media in 24 hours at room temperature. It grows well at temperatures ranging from 10 to 30° C., but poorly at 37° C. The pH range for the growth of the yeast was found to be between 3.0 and 8.0. The yeast was able to tolerate sodium chloride in a concentration ranging from 0 to 15% and to utilize glucose, lactose and lactates.

In an experiment where glucose, lactose and sodium lactate were used as sources of equivalent amounts of carbon in an otherwise complete medium adjusted to pH 4.7, the growth of the yeast for 4 days at 30° C. resulted in a shift of the pH of the medium to 3.5, 3.0 and 6.3 for glucose, lactose and sodium lactate, respectively. This is significant because in brick cheese the yeast probably metabolises the lactate, causes a shift in the pH of the medium towards neutrality and thus favors the growth of *B. linens*. It was found that *B. linens* would not grow well in media having a reaction more acid than pH 5.5.

The pure culture of the yeast then was grown for about 7 days in a lactate

Received for publication January 19, 1949.

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

² Government of India Scholar, Present address: Dairy Bacteriologist, Indian Dairy Research Institute, Bangalore.

broth and filtered through a Seitz filter. The filtrate, containing products of the activity of yeasts in the medium, was added in graduated amounts to a medium which by itself did not support the growth of *B. linens*. Upon inoculation and incubation of the medium at 30° C., the addition of the yeast filtrates markedly stimulated the growth of *B. linens*. This stimulation of growth was confirmed by means of turbidimetric observations with a Klett-Summerson colorimeter. Therefore the yeast in brick cheese may be considered to stimulate the growth of *B. linens*, probably by providing the bacterium with some accessory growth factors.

SUMMARY

The role of the yeast in brick cheese seems to be as follows:

1. The yeast metabolises the lactates in the cheese, causing a shift of pH towards neutrality. This favors the growth of *Bacterium linens*, which is unable to grow in an acid environment.
2. In addition, the yeast supplies *B. linens* with some essential growth factor, or factors.

ACKNOWLEDGMENT

We wish to thank Dr. W. V. Price for his helpful suggestions during the course of this work.

REFERENCES

- (1) LANGHUS, W. L., PRICE, W. V., SOMMER, H. H., AND FRAZIER, W. C. The Smear of Brick Cheese and its Relation to Flavor Development. *J. Dairy Sci.*, **28**, 11: 827-838. 1945.
- (2) SKINNER, C. E., EMMONS, C. W., AND TSUCHIYA, H. M. *Henrici's Yeasts, Molds and Actinomycetes*. 2nd ed. John Wiley & Sons, Inc., N. Y. 1947.

ASSOCIATION ANNOUNCEMENTS

PROGRAM FORTY-FOURTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION UNIVERSITY OF MINNESOTA MINNEAPOLIS AND ST. PAUL, MINNESOTA JUNE 21-23, 1949

PROGRAM COMMITTEE

GENERAL:

P. H. TRACY, Illinois
Chairman
J. B. FITCH, Minnesota

EXTENSION:

G. HEEBINK, West Virginia
Chairman
RAY ALBRECHTSEN, New York
H. R. SEARLES, Minnesota
C. W. REAVES, Florida

MANUFACTURING:

E. M. BARKER, Minnesota
Chairman
D. V. JOSEPHSON, Pennsylvania
J. H. HETRICK, Illinois

PRODUCTION:

L. A. MOORE, Beltsville, Md.
Chairman
G. H. WISE, North Carolina
G. M. CAIRNS, Maryland

GENERAL PROGRAM

Monday, June 20, 1949

12:00 Noon Open for Registration, Coffman Memorial Union
7:00- 9:30 Registration and Informal Gathering, Coffman Memorial
 Union

Tuesday, June 21, 1949

8:00 REGISTRATION
9:30-12:00 OPENING SESSIONS, *Main Ballroom, Coffman Memorial Union*
 J. B. FITCH, *Dairy Division, University of Minnesota,*
 presiding
 Address of Welcome
 J. L. MORRILL, *President, University of Minnesota*
 Presidential Address
 W. E. PETERSEN, *Dairy Division, University of Min-*
 nesota
 Cholesterol and the Problem of Aging
 ANCEL KEYS, *Director of Laboratory of Physiological*
 Hygiene, University of Minnesota

- 1:30- 4:30 **SECTIONAL MEETINGS**
Production Section A
 Genetics and Endocrine Investigations
Room 313, Zoology Bldg.
Production Section B
 Calf Problems
Room 06, Botany Bldg.
Manufacturing Section
 Cream, Dry Milk, Ice Cream, Sherbets
Auditorium, Museum of Natural History
Extension Section
 Dairy Herd Improvement Associations
Room 320, Coffman Memorial Union
- 4:30 **TOUR OF LAND O'LAKES CREAMERIES, INC., PLANT AND OFFICES**
- 6:00 **DINNER AND ENTERTAINMENT**
 Courtesy of Land O'Lakes Creameries, Inc.
Wednesday, June 22, 1949
- 9:00-12:00 **SECTIONAL MEETINGS**
Production Section A
 Temperature Effects, Vitamins
Room 06, Botany Bldg.
Production Section B
 Forages, Growth, Metabolism
Room 313, Zoology Bldg.
Manufacturing Section
 Symposium on Milk Proteins
Auditorium, Museum of Natural History
Extension Section
 Teaching Methods and Exhibits
Room 320, Coffman Memorial Union
- 1:00 **ASSOCIATION PHOTOGRAPH**
- 1:30- 4:00 **SECTIONAL MEETINGS**
Production and Extension Sections
Auditorium, Physics Bldg.
 Panel Discussion—The Job of Herd Improvement
Manufacturing Section
 Chemistry
Auditorium, Museum of Natural History
- 4:15 **"BRUCELLOSIS IN MAN"**
 W. W. SPINK, *Professor of Medicine, University of Minnesota.* Auditorium, Physics Bldg.
- 5:00- 5:30 **COMMITTEE AND BUSINESS MEETINGS**

8:00 **RECEPTION AND DANCE**
Ballroom, Coffman Memorial Union

Thursday, June 23, 1949

9:00-12:00 **SECTIONAL MEETINGS AND BUSINESS MEETINGS**
Production Section A
 Mammary Secretion, Mastitis
Room 313, Zoology Bldg.
Production Section B
 Semen Techniques
Room 06, Botany Bldg.
Manufacturing Section
 Chemistry, Microbiology, Standards
Auditorium, Museum of Natural History
Extension Section
 4-H Club Work and Committee Reports
Room 320, Coffman Memorial Union

1:30- 3:00 **SECTIONAL MEETINGS**
Production Section A
 Feeding and Management
Room 313, Zoology Bldg.
Production Section B
 Semen Metabolism, Reproduction
Room 06, Botany Bldg.
Manufacturing Section
 Dry Milk, Cheese
Auditorium, Museum of Natural History
Extension Section
 Dairy Breeding
Room 320, Coffman Memorial Union

3:00- 5:00 **BUSINESS MEETING OF THE ASSOCIATION**
Auditorium, Museum of Natural History

7:00 **ANNUAL BANQUET, INSTALLATION OF OFFICERS AND PRESEN-**
TATION OF AWARDS. *Ballroom, Coffman Memorial Union*

PROGRAM OF ENTERTAINMENT

(Principally for the Ladies)

Tuesday, June 21, 1949

1:00 ***LUNCHEON, Radisson Hotel, Minneapolis.**
 Courtesy of Milk Dealers of the Twin Cities, Ice Cream
 Manufacturers, and Twin City Milk Producers
 Association.
4:30 **TOUR, Land O'Lakes Creameries, Inc.**

- 6:00 DINNER AND ENTERTAINMENT (Open to all registered)
Courtesy of Land O'Lakes Creameries, Inc.

Wednesday, June 22, 1949

- 12:00 *LUNCHEON AND ENTERTAINMENT. Nicollet Hotel, Minneapolis; Entertainment by Home Service Department in Betty Crocker's Kitchens. Courtesy of General Mills, Inc.

- 8:00 RECEPTION AND DANCE, Coffman Memorial Union. (Open to all registered)

Thursday, June 23, 1949

- 1:00- 4:00 *BRIDGE AND TEA, Coffman Memorial Union.

- 7:00 BANQUET, INSTALLATION AND PRESENTATION OF AWARDS.
Ballroom, Coffman Memorial Union. (Open to all registered)

* Open to ladies only.

PROGRAM OF MANUFACTURING SECTION

Tuesday, June 21

Afternoon Session. *Auditorium, Museum of Natural History*

- 1:30- 4:30 CREAM, DRY MILK, ICE CREAM, SHERBETS. E. M. BARKER, *Chairman*.
- M1 Body of Cultured Cream. E. S. GUTHRIE, *Cornell University*.
- M2 The Anti-oxidant Properties of Nordihydroguaiaretic Acid in Cream. VLADIMIR N. KRUKOVSKY, DIONISIOS A. THEOKAS AND FRANK A. WHITING, *Cornell University*.
- M3 The Relation Between the Degree of Solidification of Fat in Cream and its Churning Time. J. ROBERT BRUNNER, *Michigan State College*, AND E. L. JACK, *University of California*.
- M4 The Stability to Drying of Added Vitamin A to Spray Dried Milk. FLOYD C. OLSON, GEORGE W. GRUBER, ROBERT KOZLIK AND KERMIT BROWN, *Maple Island Farm, Inc.*
- M5 The Effect of Variations in Acidity on the Keeping Quality of Dried Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *U.S.D.A.*
- M6 A Method of Measuring Ice Crystal and Air Cell Size of Ice Cream by Microscopical Examination. L. F. BLANTON AND W. S. ARBUCKLE, *North Carolina State College*.

- M7 The Use of Whey in Sherbets. F. E. POTTER AND D. H. WILLIAMS, *U.S.D.A.*
- M8 The Effect of Some Emulsifying Agents on the Physical-Chemical Properties of Ice Cream. J. J. SHEURING, *University of Georgia*, HARRY PYENSON AND P. H. TRACY, *University of Illinois*.
- M9 Some Factors Influencing Shrinkage in Ice Cream. JOHN J. SHEURING, *University of Georgia*.
- M10 The Manufacture of "Cultured" Ice Cream. W. H. E. REID, J. H. GHOLSON, C. B. AGEE AND R. M. HANCKEL, *Missouri Agricultural Experiment Station*.
- M11 Utilization of Dehydrated Whey Solids in Ice Creams and Sherbets. J. H. GHOLSON, W. H. E. REID, R. J. BASNETT AND R. M. HANCKEL, *Missouri Agricultural Experiment Station*.
- M12 The Relative Sweetness of Certain Corn Sweeteners in Ice Cream. L. D. HILKER, *National Dairy Research Laboratories, Inc.*

Wednesday, June 22

Morning Session. Auditorium, Museum of Natural History

9:00-12:00 **SYMPOSIUM ON MILK PROTEINS.** J. H. HETRICK, *Chairman*.

Important Considerations in Protein Chemistry Research. A. M. SWANSON, *University of Wisconsin*.

Crystalline Proteins from Milk Serum. H. KLOSTERGAARD AND J. D. INGLE, *Swift & Co., Chicago*.

Discussion Leader: R. McL. WHITNEY, *University of Illinois*.

Some Effects of Heat Treatment on the Serum Proteins of Milk. R. JENNESS, *University of Minnesota*.

Discussion Leader: P. G. MILLER, *Carnation Co., Milwaukee*.

Some Chemical and Physical Characteristics of Alpha and Beta Casein. E. C. HAGBERG AND A. M. SWANSON, *University of Wisconsin*.

Discussion Leader: Z. D. ROUNDY, *Armour & Co., Chicago*.

Wednesday, June 22

Afternoon Session

1:00 **ASSOCIATION PHOTOGRAPH.**

1:30- 4:15 **CHEMISTRY.** D. V. JOSEPHSON, *Chairman. Auditorium, Museum of Natural History.*

- M13 The Sizes of the Colloidal Protein Particles of Skim Milk. T. F. FORD AND G. A. RAMSDALL, *Bureau of Dairy Industry, U.S.D.A.*
- M14 Determination of Reducing Groups in Proteins and in Milk with *o*-iodosobenzoate. BRUCE LARSON AND ROBERT JENNESS, *University of Minnesota.*
- M15 Isolation of Minor Organic Compounds From Heated Milk. STUART PATTON AND DAVID G. KEENEY, *Pennsylvania State College.*
- M16 Milk Surfaces. II. Surface Tension Changes in Relation to Some Treatments of Milk. C. H. WHITNAH AND W. H. CHILSON, *Kansas Agricultural Experiment Station.*
- M17 Turbidity as a Means for Determining the Efficiency of Homogenization. U. S. ASHWORTH, *State College of Washington.*
- M18 The Instability of Ascorbic Acid in Water, with Added Copper or Hydrogen Peroxide or Both. R. W. BELL AND T. J. MUCHA, *Bureau of Dairy Industry, U.S.D.A.*
- M19 Deferment of an Oxidized Flavor in Frozen Milk by Ascorbic Acid Fortification and by Hydrogen Peroxide Oxidation of the Ascorbic Acid of the Fresh Milk. R. W. BELL AND T. J. MUCHA, *Bureau of Dairy Industry, U.S.D.A.*
- M20 A Re-evaluation of the Hortvet Formula and Freezing Point Value of Milk in Estimating the Percentage of Added Water. W. A. KRIENKE AND L. R. ARRINGTON, *University of Florida.*
- M21 Electrometric Titration of Milk and Dairy Products in the Determination of Titratable Acidity. W. A. KRIENKE, *University of Florida.*
- M22 Preliminary Observations of the Effects of Ladino Pasture and Hay Feeding on Tocopherol Content of the Fat and Stability of Milk. VLADIMIR N. KRUKOVSKY, J. K. LOOSLI AND DIONISIOS A. THEOKAS, *Cornell University.*
- M23 Effects of External Temperature and Pasturage on the Degree of Unsaturation of Milk Fat. E. E. BARTLEY, E. W. BIRD, C. Y. CANNON AND J. H. ZALETEL, *Iowa State College.*
- M24 Separation of Fatty Acids by Displacement Chromatography and its Application to Analysis of Butter Fat. RALPH T. HOLMAN AND LENNART HAGDAHL, *Texas A. and M. College.*

M25 Some Observations on Fat Fractions from Butter Oil.
ARTHUR T. MUSSETT, STUART PATTON AND CHESTER D.
DAHLE, *Pennsylvania State College.*

4:15- 5:00 **GENERAL SESSION.** *Auditorium, Physics Bldg.*
Brucellosis in Man, W. W. SPINK, *Professor of Medicine,*
University of Minnesota.

5:00- 5:30 **SECTION BUSINESS MEETING.** *Auditorium, Mu-*
seum of Natural History.

Thursday, June 23

Morning Session. Auditorium, Museum of Natural History

9:00-11:00 **CHEMISTRY, MICROBIOLOGY, STANDARDS.** J.
H. HETRICK, *Chairman.*

M26 The Steam Distillation of Stale-flavor Component
from Stale Butteroil. R. McL. WHITNEY, KATHERINE
PAULSON AND P. H. TRACY, *University of Illinois.*

M27 The Extraction of Stale Butteroil from Stale Dried
Whole Milk by Organic Solvents. R. McL. WHITNEY
AND P. H. TRACY, *University of Illinois.*

M28 Sanitary Standardization of Equipment Used in the
Dairy Industry. E. H. PARFITT, *Evaporated Milk*
Association.

M29 Nutrition of the Lactic Group of Streptococci and its
Relation to Bacteriophage Multiplication. E. B.
COLLINS, F. E. NELSON AND C. E. PARMELEE, *Iowa*
Agricultural Experiment Station.

M30 Thermal Death Time Studies of Coliform Bacteria in
Milk. J. C. OLSON, JR., H. MACY AND H. O. HALVOR-
SON, *University of Minnesota.*

M31 Studies on Acid Production, Loss of Bacteriophage
and Resistance of a Bacteriophage-sensitive Culture
of *Streptococcus lactis*. H. F. FORD AND F. J. BABEL,
Purdue University.

M32 Variations Encountered in the Grading of Raw Milk
with the Methylene Blue and Resazurin Reduction
Tests. R. K. LEWTON, D. M. MARKLAND AND F. J.
BABEL, *Purdue University.*

M33 Standards for Grades of Milk for Use in Manufac-
tured Dairy Products. C. J. BABCOCK AND H. J.
EMERY, *Manufactured Dairy Products Division,*
U.S.D.A.

M34 The Effect of Certain Metallic Ions on Germicidal Ac-
tivity of Quaternary Ammonium Germicides. W. S.
MUELLER AND D. B. SEELEY, *University of Massachu-*
setts.

11:00-12:00 **BUSINESS MEETING.**

Thursday, June 23

Afternoon Session. Auditorium, Museum of Natural History

- 1:30- 3:00 **DRY MILK, CHEESE.** D. V. JOSEPHSON, *Chairman.*
- M35 Optimum Consumer Preference for Dry Milk in Bread. E. L. JACK AND (MRS.) V. M. HAYNES, *University of California.*
- M36 The Utilization of Roller and Spray Dried Sweet Cream Buttermilk in Bread Making. J. V. REGER, W. B. COMBS, S. T. COULTER AND R. B. KOCH, *University of Minnesota.*
- M37 The Relation of Surface Growth to the Ripening of Minnesota Blue Cheese. H. A. MORRIS, W. B. COMBS AND S. T. COULTER, *University of Minnesota.*
- M38 The Manufacture of Blue Cheese from Pasteurized Homogenized Milk. I. I. PETERS AND F. E. NELSON, *Iowa Agricultural Experiment Station.*
- M39 The Determination of Free Tryptophane in Cheese. ARTHUR B. EREKSON, *Lakeshire-Marty Company, Plymouth, Wisconsin.*
- M40 Filter Paper Chromatography as a Means to Determine the Amino Acids and Amines Developed in Cheddar Cheese During Ripening. F. V. KOSIKOWSKY, *Cornell University.*
- M41 Manufacture of Cottage Cheese from Reconstituted Non-Fat Dry Milk Solids. C. E. PARMELEE AND W. S. ROSENBERGER, *Iowa Agricultural Experiment Station.*
- 3:00- 5:00 **BUSINESS MEETING OF THE ASSOCIATION.**
Auditorium, Museum of Natural History.

PROGRAM OF PRODUCTION SECTION

Tuesday, June 21

Afternoon Session

- 1:30- 4:30 **Section A, GENETICS AND ENDOCRINE INVESTIGATIONS.** L. A. MOORE *Chairman.*
Room 313, Zoology Bldg.
- P1 Differences in Production, Type, Size and Breeding Efficiency of Cow Families. KENNETH A. TABLER, W. J. TYLER AND GEORGE HYATT, JR., *West Virginia University.*
- P2 Prolonged Gestation of Genetic Origin in Cattle. S. W. MEAD, P. W. GREGORY AND W. M. REGAN, *University of California.*

- P3 Estimation of Changes in Herd Environment. C. R. HENDERSON, *Cornell University*.
- P4 The Number of Proved Sons Necessary to Evaluate the Transmitting Ability of a Sire. W. E. WASHBON AND W. J. TYLER, *West Virginia University*.
- P5 Calf Mortality, Sex Ratio and Incidence of Twinning in Two University of Minnesota Herds. KENNETH MILLER AND L. O. GILMORE, *Minnesota Agricultural Experiment Station*.
- P6 Observations on Mammary Gland Development of Dairy Heifers Induced by Hormone Injections. J. F. SYKES, T. R. WRENN AND P. C. UNDERWOOD, *Bureau of Dairy Industry, U.S.D.A.*
- P7 Effect of Temperature and Drying on Male Hormone in Cow Manure. C. W. TURNER, *Missouri Agricultural Experiment Station*.
- P8 Effect of Mild Hyperthyroidism on Milk Production in Dairy Cattle. C. W. TURNER, *Missouri Agricultural Experiment Station*.
- P9 Effects and Economy under Tennessee Conditions of Thyroprotein Feeding during Lactation Decline. ERIC W. SWANSON, *University of Tennessee*.
- P10 Size of Thyroid in Cows from Southern States. W. W. SWETT AND C. A. MATTHEWS, *Bureau of Dairy Industry, U.S.D.A.*
- P11 Factors Affecting Heart Rates of Dairy Cows. J. W. THOMAS, *Bureau of Dairy Industry, U.S.D.A.*

1:30- 4:30 Section B. CALF PROBLEMS. G. M. CAIRNS, *Chairman. Room 06, Botany Bldg.*

- P12 Milk Substitutes for Young Dairy Calves. H. D. WALLACE, J. K. LOOSLI AND K. L. TURK, *Cornell University*.
- P13 Milk Replacements in the Rations of Dairy Calves. J. B. WILLIAMS AND C. B. KNOTT, *Pennsylvania State College*.
- P14 Diurnal Variations in Concentrations of Fat in Blood Plasma of Calves Fed Various Types of Oils. H. B. BARKER AND N. L. JACOBSON, *Iowa State College*.
- P15 The Hydrogen Ion Concentration and Dry Matter of the Feces of Young Dairy Calves Raised on a Limited Whole Milk-Dry Starter Method. R. E. JOHNSON, H. D. EATON, J. H. KRAMER, E. L. JUNGHER, W. N. PLASTRIDGE AND L. NEZVESKY, *University of Connecticut and Storrs Agricultural Experiment Station*.

- P16 The Influence of Pasture and Rumen Inoculation on the Establishment of Certain Microorganisms in the Rumens of Young Dairy Calves. W. D. POUNDEN AND J. W. HIBBS, *Ohio Agricultural Experiment Station.*
- P17 The Influence of Pasture and Early Rumen Development on the Changes in the Plasma Carotenoids, Vitamin A and Ascorbic Acid and the Liver Storage of Carotenoids and Vitamin A of Young Dairy Calves. J. W. HIBBS AND W. D. POUNDEN, *Ohio Agricultural Experiment Station.*
- P18 Carotene Requirements for Young Dairy Calves. R. F. ELLIOTT, *Cornell University.*
- P19 The Plasma Levels of Carotene and Vitamin A in Calves from Dams Milked Prepartum and in Calves from Dams Milked Postpartum. H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON AND L. D. MATTERSON, *University of Connecticut and Storrs Agricultural Experiment Station.*
- P20 Effect of Type of Dispersion on Rate of Absorption of Carotene and Vitamin A by Dairy Calves. G. H. WISE, N. L. JACOBSON, R. S. ALLEN AND S. P. YANG, *Iowa State College.*
- P21 Studies on the Site of Absorption and Conversion of Carotene to Vitamin A in the Dairy Calf. R. F. ELLIOTT, *Cornell University.*
- P22 Calf Losses in a Dairy Herd Consisting of Five Breeds. E. E. ORMISTON, *University of Illinois.*

Wednesday, June 22

Morning Session

- 9:00-12:00 Section A, **TEMPERATURE EFFECTS, VITAMINS.**
L. A. MOORE, *Chairman.*
Room 06, Botany Bldg.
- P23 The Influence of Variations in Environmental Temperature and Thyroid Status on Sexual Development in the Male Mouse. M. MAQSOOD AND E. P. REINEKE, *Michigan State College.*
- P24 Factors Affecting Heat Tolerance of Dairy Cattle. R. E. McDOWELL AND R. A. HILDER, *Bureau of Dairy Industry, U.S.D.A.*
- P25 The Comparative Heat Tolerance of Red Sindhi X Jersey and Other Breeds of Dairy Calves. R. A. HILDER AND R. E. McDOWELL, *Bureau of Dairy Industry, U.S.D.A.*

- P26 Reactions of Dairy Cows to Higher Temperatures. SAMUEL BRODY, *Missouri Agricultural Experiment Station.*
- P27 The Effect of Increasing Environmental Temperatures on the Composition of Milk. J. W. COBBLE AND A. C. RAGSDALE, *University of Missouri.*
- P28 The Influence of Temperature on the Carotenoid and Vitamin A Content of Milk Fat. O. T. STALLCUP AND A. C. RAGSDALE, *University of Missouri.*
- P29 The Carotene Requirements of Guernsey Cattle for Reproduction. A. H. KUHLMAN AND W. D. GALLUP, *Oklahoma A. and M. College.*
- P30 Vitamin A Absorption Studies in Ruminants. R. P. NIEDERMEIER, VEARL R. SMITH AND L. H. SCHULTZ, *University of Wisconsin.*
- P31 Relation Between the Carotene in the Feed and the Vitamin A Potency of Butter. H. G. WISEMAN AND J. B. SHEPHERD, *Bureau of Dairy Industry, U.S.D.A.*
- P32 Further Studies on the Relation of Soybeans to the Vitamin A Requirements of Dairy Cattle. M. F. ELLMORE AND J. C. SHAW, *University of Maryland.*
- P33 Role and Sources of B₁₂ in the Normal Mammal. A. M. HARTMAN, L. P. DRYDEN AND C. A. CARY, *Bureau of Dairy Industry, U.S.D.A.*
- P34 The Vitamin D Content of Roughages. G. C. WALLIS, C. A. SMITH AND R. H. FISHMAN, *Standard Brands, New York, N. Y., and Agricultural Experiment Stations of Florida, Illinois, Kansas, Michigan, New York, North Carolina, South Dakota, Texas and Washington, and the California State Polytechnic College.*

9:00-12:00 Section B, **FORAGES, GROWTH, METABOLISM.**
 G. M. CAIRNS, *Chairman.*
Room 313, Zoology Bldg.

- P35 The Effect of the Proportion of Roughage in the Ration on the Growth of Dairy Heifers. K. E. HARSHBARGER AND G. W. SALISBURY, *University of Illinois.*
- P36 Preliminary Report on the Influence of Soil Fertility on the Health, Reproduction and Milk Production of Dairy Cows. C. W. DUNCAN, K. M. DUNN, R. E. ELY, S. T. DEXTER AND C. E. MILLAR, *Michigan Agricultural Experiment Station.*
- P37 Conservation of Nutrients and Feeding Value of Wilted Silage, Barn-Cured Hay and Dehydrated Hay. R. E. ELY, L. G. SCHOENLEBER, J. B. SHEPHERD, H. G.

- WISEMAN, C. G. MELIN, W. H. HOSTERMAN AND R. E. WAGNER, *Bureau of Dairy Industry, Bureau of Plant Industry, Soils, and Agricultural Engineering, and Production and Marketing Administration, U.S.D.A.*
- P38 Observations on Time Required for Dairy Cows to Eat Grain, Silage and Hay. K. E. HARSHBARGER, *University of Illinois.*
- P39 How Hay Feeding to Cows on Pasture Affected Milk Production and Body Weight. DWIGHT M. SEATH, *University of Kentucky.*
- P40 A Method for Estimating the Feed-Replacement Value of Pasture Forage. W. B. NEVENS, R. W. TOUCHBERRY AND J. A. PRESCOTT, JR., *University of Illinois.*
- P41 Distribution of Intravenously Injected Radioactive Phosphorous (P32) in the Body of the Dairy Cow. N. P. RALSTON, MAX KLEIBER, A. H. SMITH AND A. L. BLACK, *University of California.*
- P42 The Effects of Lactose Feeding on Lactase Production. JESSIE FISCHER, T. S. SUTTON, J. L. LAWRENCE, H. H. WEISER AND G. L. STAHLY, *Ohio State University.*
- P43 Blood Sugar Studies in Relation to Ketosis in Ruminants. L. H. SCHULTZ, VEARL R. SMITH AND H. A. LARDY, *University of Wisconsin.*
- P44 Biochemical and Histo-Pathological Studies of Fasting Ketosis and Spontaneous Ketosis of Cows. J. C. SHAW, P. V. SAARINEN, B. C. HADJIOLOS AND E. C. LEFFEL, *University of Maryland.*
- P45 Standards for Growth in Weight of Jersey Heifers. C. A. MATTHEWS AND M. H. FOHRMAN, *Bureau of Dairy Industry, U.S.D.A.*
- P46 The Value of Wood Molasses for Growth of Dairy Heifers. T. H. BLOSSER, G. W. SCOTT, R. E. ERB AND A. O. SHAW, *State College of Washington.*

Wednesday, June 22

Afternoon Session. Auditorium, Physics Bldg.

1:00 ASSOCIATION PHOTOGRAPH.

1:30- 3:15 JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS.

L. A. MOORE AND G. HEEBINK, *Co-Chairmen.*

PANEL DISCUSSION—The Job of Herd Improvement. JOE TAYLOR, *Pennsylvania State College, Leader.*

1. Allowing for the Effect of Environment on Production. E. E. HEIZER, *University of Wisconsin.*

2. Estimating the Breeding Value of Young Bulls.

J. L. LUSH, *Iowa State College.*

3. Should a Bull be Linebred or Out bred?

G. A. BOWLING, *Farm Manager, formerly from West Virginia University.*

4. What about Indexes in the Selection of Bulls?

V. A. RICE, *University of Massachusetts.*

5. Results from Crossbreeding.

MILTON FOHRMAN, *Bureau of Dairy Industry, U.S.D.A.*

6. Reasonable Production Increase to be Expected from Culling.

FRANK ASTROTH, *Jersey Breeder, St. Paul, Minnesota.*3:15- 4:15 **JOINT COMMITTEE REPORTS.**Breeds Relations Committee. H. A. HERMAN, *Chairman.*
Dairy Cattle Health Committee. W. E. PETERSEN, *Chairman.*Dairy Cattle Breeding Committee. E. J. PERRY, *Chairman.*
Type Classification Committee. J. W. LINN, *Chairman.*4:15- 5:00 **GENERAL SESSION.***Brucellosis in Man.* W. W. SPINK, *Professor of Medicine, University of Minnesota.*5:00- 5:30 **COMMITTEE MEETINGS***Thursday, June 23**Morning Session*9:00-11:00 Section A, **MAMMARY SECRETION, MASTITIS.**
L. A. MOORE, *Chairman.*
*Room 313, Zoology Bldg.*P47 Effect of Various Milking Procedures, Prepartum and Postpartum, on Composition of Mammary Secretions.
D. B. PARRISH, F. C. FOUNTAINE, G. H. WISE, F. W. ATKESON AND J. S. HUGHES, *Kansas Agricultural Experiment Station.*P48 Some Effects of Prepartum Milking on the Performance of Cows and Calves. R. A. ACKERMAN, GEORGE HYATT, JR. AND A. H. VAN LANDINGHAM, *West Virginia University.*P49 The Effect of Prepartum Milking on the Ascorbic Acid and Riboflavin Content of Colostrum at Parturition.
A. H. VAN LANDINGHAM, C. A. FLANDERS AND R. A. ACKERMAN, *West Virginia University.*

P50 Effectiveness of Penicillin Infusions in Eliminating Mastitis Infections in the Bureau of Dairy Industry Herd. W. W. SWETT, L. A. BURKEY, CECELIA R.

BUCKNER AND P. C. UNDERWOOD, *Bureau of Dairy Industry, U.S.D.A.*

- P51 The Incidence and Relative Severity of Infections of Different Organisms in Mastitis. LLOYD A. BURKEY AND CECILIA R. BUCKNER, *Bureau of Dairy Industry, U.S.D.A.*
- P52 A Study of the Reliability of Various Diagnostic Tests and the Efficiency of Certain Therapeutic Measures in the Control of Mastitis. C. P. MERILAN, H. A. HERMAN, J. E. EDMONDSON, K. L. TALLMAN AND O. S. CRISLER, *University of Missouri.*
- P53 Preliminary Observations on the Biochemical and Serological Characteristics of Coliform Organisms Isolated From Cases of Acute Mastitis. J. C. OLSON, JR., I. A. SCHIPPER AND M. E. SCHMITZ, *University of Minnesota.*
- P54 Comparison of the Incidence and Severity of Mammary Edema of Cows Fed Roughages Alone or Roughages Plus Grain during the Dry Period. F. C. FOUNTAINE, D. B. PARRISH AND F. W. ATKESON, *Kansas Agricultural Experiment Station.*

9:00-11:00 Section B, **SEMEN TECHNIQUES.** G. M. CAIRNS, *Chairman.*

Room 06, Botany Bldg.

- P55 Diluting Bull Semen on the Basis of Numbers of Spermatozoa Rather than by Volume. CECIL BEANTON, M. H. NEWSOM AND T. E. PATRICK, *Louisiana State University.*
- P56 Penicillin and Sulfanilamide in Semen Dilutors and Their Effect on Fertility of Semen from Relatively Fertile Bulls. JOHN P. MIXNER, *New Jersey Agricultural Experiment Station.*
- P57 A Comparison of Penicillin, Streptomycin and Sulfanilamide for Improving the Fertility of Semen from Relatively Infertile Bulls. JOHN O. ALMQUIST, *Pennsylvania State College.*
- P58 Fertility of Bull Semen Diluted from 1:100 to 1:300. E. L. WILLETT, *American Foundation for the Study of Genetics.*
- P59 Buffered Whole Egg as a Nutrient Extender for Bovine Spermatozoa. H. O. DUNN AND R. W. BRATTON, *Cornell University.*
- P60 The Fertility of Bovine Semen Cooled with and without the Addition of Citrate-Sulfanilamide-Yolk Ex-

tender. R. H. FOOTE AND R. W. BRATTON, *Cornell University*.

P61 Relation of the Eosin-Aniline Blue Staining Method to the Quality of Bull Semen. H. E. SHAFFER AND J. O. ALMQUIST, *Pennsylvania State College*.

P62 The Effect of Frequency of Collection Upon Semen Production and Fertility of Dairy Bulls used in Artificial Breeding. T. E. PATRICK, CECIL BRANTON AND M. H. NEWSOM, *Louisiana State University*.

11:00-12:00 **PRODUCTION SECTION BUSINESS MEETING.**
Room 06, Botany Bldg.

Thursday, June 23

Afternoon Session

1:30- 3:00 Section A, **FEEDING AND MANAGEMENT.** L. A. MOORE, *Chairman.*
Room 313, Zoology Bldg.

P63 Clipping as an Aid to Control of Cattle Lice. R. B. PRICE, JR., W. C. PRIGGE, N. N. ALLEN AND R. J. DICKE, *University of Wisconsin*.

P64 The Effect of Methods of Milking, Methods of Cooling the Milk and Types of Barns on the Total Bacteria Count and Coliform Count. C. C. FLORA, P. M. REAVES AND C. W. HOLDAWAY, *Virginia Agricultural Experiment Station*.

P65 Some Observations on Recovery in Dairy Production in Western Europe. W. H. RIDDELL, *University of Vermont*.

P66 Feeding Value of Dehydrated Sweet Potatoes Fed Wet as Compared with Corn-Soybean Silage for Lactating Cows. I. L. RUSOFF, B. J. BURCH, JR., J. B. FRYE, JR., AND G. D. MILLER, *Louisiana State University*.

P67 Effect of Excess Concentrate Feed Consumption on Milk Production of Dairy Cows in Hawaii. L. A. HENKE, *Hawaii Agricultural Experiment Station*.

P68 Influence of Various Udder Treatments Upon the Let-Down of Milk. C. E. KNOOP AND C. F. MONROE, *Ohio Agricultural Experiment Station*.

P69 A Comparison of Milk Production Between the Prepartum Milked Halves and the Non-prepartum Milked Halves of Bovine Udders. M. L. DAWDY AND C. B. KNOTT, *Pennsylvania State College*.

1:30- 3:00 Section B, **SEMEN METABOLISM, REPRODUCTION.**
G. M. CAIENS, *Chairman.*
Room 06, Botany Bldg.

- P70 The Effect of in Vitro Treatments with Testosterone on the Oxygen Consumption of Ejaculated Spermatozoa. F. N. BAKER, A. B. SCHULTZE AND H. P. DAVIS. *University of Nebraska.*
- P71 Complementary Effect of Acetylcholine and Thyroxine on O₂ Consumption of Bovine Semen. A. B. SCHULTZE, *University of Nebraska.*
- P72 Recovery of the Fertilized Ovum from the Living Cow. ARTHUR E. DRACY, *South Dakota State College,* AND W. E. PETERSEN, *University of Minnesota.*
- P73 Factors Affecting the Interval Between Parturition and Subsequent Estrus in Dairy Cattle. J. H. EDMONDSON, *University of Missouri.*
- P74 Comparison of pH Values of in Vivo and in Vitro Determinations on Bovine Vaginal-Cervical Mucus. D. B. ROARK AND H. A. HERMAN, *University of Missouri.*
- P75 The Interrelationship of Age and Season on Bull Fertility. T. M. LUDWICK, D. S. RUDRAIAH, JAMES ROSENBERGER AND FORDYCE ELY, *Ohio Agricultural Experiment Station.*

3:00- 5:00 **BUSINESS MEETING OF THE ASSOCIATION.**
Auditorium, Museum of Natural History.

PROGRAM OF EXTENSION SECTION

Tuesday, June 21

Afternoon Session. Room 320, Coffman Memorial Union.

- 1:30- 4:30 **Opening Business Section and Dairy Herd Improvement Associations.** G. HEEBINK, *Chairman.*
- E1 Suggested Revisions of the DHIA Herd Book. J. F. KENDRICK, *Bureau of Dairy Industry, U.S.D.A.*
- E2 Comparison of DHIA Computing Tables. C. R. GEARHART, *Pennsylvania State College.*
- E3 Progress Report on Use of I.B.M. Machines in Processing DHIA Records. H. C. GILMORE, *Pennsylvania State College.*
- E4 Use of I.B.M. Equipment for more Efficient Processing of BDI 718 Reports. RAYMOND ALBRECHTSEN, *Cornell University.*
- E5 Centering Date Versus Calendar Month for Computing Production Records. ROGER MORRISON AND R. E. ERB, *Washington State College.*

*Wednesday, June 22***Morning Session. Room 320, Coffman Memorial Union.**

- 9:00-12:00 Teaching Methods and Exhibits. C. W. REAVES, *Chairman.***
E6 Extension Education on Milking Machine Operation.
I. E. PARKIN, *Pennsylvania State College.*
E7 Development of a Successful Integrated Dairy Program.
E. C. SCHEIDENHELM, *Rutgers University.*
E8 The Michigan Program of Brucellosis Control in Cattle.
R. E. HORWOOD, *Michigan State College.*
Explanation and Discussion of Exhibits. HILTON BOYNTON, *University of New Hampshire, in charge.*

*Wednesday, June 22***Afternoon Session**

- 1:00 ASSOCIATION PHOTOGRAPH**
1:30- 4:15 Joint Meeting of Extension and Production Sections.
See Production Section Program. *Auditorium, Physics Bldg.*
4:15- 5:00 General Session
Brucellosis in Man.* W. W. SPINK, *Professor of Medicine,
University of Minnesota. Auditorium, Physics Bldg.

*Thursday, June 23***Morning Session. Room 320, Coffman Memorial Union**

- 9:00-12:00 4-H Club Work, Committee Reports and Business Meeting.**
G. HEEBINK, *Chairman.*
E9 4-H Show Programs as Developed in Mississippi.
L. A. HIGGINS, *Mississippi State College.*
E10 Training 4-H Dairy Project Leaders. E. T. ITSCHNER,
M. J. REGAN AND W. H. CLONINGER, *University of Missouri.*
Committee Reports
Business Meeting

*Thursday, June 23***Afternoon Session. Room 320, Coffman Memorial Union.**

- 1:30- 3:00 Dairy Breeding. G. HEEBINK, *Chairman.***
E11 Analysis of Production Records of the Daughters of Sires Used in the New York Artificial Insemination Program.
RAYMOND ALBRECHTSEN, *Cornell University.*
E12 A Different Slant on Sire Selection. W. E. WASHBON,
West Virginia University.
3:00- 5:00 Business Meeting of the Association. *Auditorium, Museum of Natural History.*

NUMBER 6

Lithographic Lithography

Various other effects not directly related to fertility and reproduction have been attributed to vitamin E in the nutrition of ruminants. Willman *et al.* (28) reported success in treating a characteristic muscular syndrome of lambs, known as "stiff-lamb" disease with wheat germ oil, *alpha*-tocopherol or mixed tocopherols. Recently it was shown by Gullickson and Calverley (9) that cattle fed vitamin E-deficient rations may die from heart failures. Harris *et al.* (13) reported that when dairy cows are fed a daily supplement of 1 g. of mixed natural tocopherols the fat output is increased by about 25 per cent; this contention, however, has not been confirmed by others (10, 24, 27).

The present experiment was designed to determine whether vitamin E is needed by cattle for successful reproduction. The plan followed was to feed all animals throughout their lives on rations containing liberal amounts of all known essential nutritional factors except vitamin E. The relationship of vitamin E to growth, health and milk production of cattle also is considered.

EXPERIMENTAL PROCEDURE

Selection of feedstuffs. Before any cattle were started on the experiment, suitable feeds low or completely lacking in vitamin E had to be found. This was accomplished by conducting rat bioassays on a great variety of feedstuffs such as commonly are fed to cattle. A modification of the bioassay method of Palmer (22) was followed. Sexually mature female rats that had been fed normal rations throughout the period of growth and their first gestation were placed on the basal vitamin E-free ration immediately after the birth of their litters. After weaning at 21 to 25 days the young were reared to sexual maturity on the basal vitamin E-free ration modified so as to contain such percentage of the feedstuff tested as was likely to be present, on the dry matter basis, when fed in maximum amounts to cattle on experiment. Each feedstuff was tested in this manner and some were tested again in combination with others to determine their additive effect, if any, on reproduction. If the product was highly indigestible for rats, a benzene extract of it was made and this then was incorporated at high levels in the basal vitamin E-free ration which was fed during the gestation period of the vitamin E-deficient rats. The rats were mated when about 90 days old and thus received the test food for periods of 9 to 10 weeks. Wheat germ oil of known potency was employed for the positive controls, a total of 3.5 to 4.0 ml. being incorporated, in the basal ration over a period of 6 to 7 weeks, corresponding to the minimum period the test feeds were consumed. At the start of the experiment test rats were allowed to litter normally, but later, to permit better control, the animals were sacrificed on the 21st day of pregnancy and the living and dead young and the resorptions were counted *in utero*. A less complete study was made with male rats of the ability of some of the products to prevent the characteristic testicular degeneration. The results of these tests provide confirmatory evidence of vitamin E content.

Table 1 lists the feeds which gave less than 100 per cent live litter efficiency when tested. The feedstuffs commonly fed to cattle were found to be relatively

rich in vitamin E, as shown by the following comparatively small amounts needed to give 100 per cent live litter efficiency: alfalfa hay, 114 g.; prairie hay, 135 g.; reed canary grass hay, 147 g.; oat hulls, 132 g.; wheat straw, 131 g.; corn bran, 133 g.; wheat gluten, 40 g.; and fish meal, 59 g. These results are in agreement with those reported by Cabell and Ellis (5).

After the ingredients in the ration of the cattle had been selected, tests were made on all new lots of feed acquired. If vitamin E was indicated in any of them or in the ration in which they were included, the feedstuff responsible either was discarded or was subjected to such treatment as had been found to inactivate the vitamin E present. Thus the vitamin E present in some lots of rice straw was inactivated subsequently by heat treatment at 90 to 100° C. for

TABLE 1
A comparison of the vitamin E activity of various feedstuffs

Feedstuff tested	Average amount consumed	No. of female rats used	No. of complete resorptions	No. of live litters	Live litters
	(g.)				(%)
Brewers' dried grains, heated 2×	310	6	5	1	16.7
Brewers' dried grains, heated 3×	414	10	10	0	0
Corn starch	150	6	6	0	0
Corn gluten meal	123	24	13	11.	45.8
Distillers' grains, solvent extracted	191	6	6	0	0
Dry skim milk	483	10	10	0	0
Meat scraps	121	7	4	3	42.9
Molasses beet pulp	360	10	6	4	40.0
Plain beet pulp	112	15	4	11	73.3
Polished rice	367	8	7	1	12.5
Polished rice, heat treated	730	8	8	0	0
Potato meal	426	10	7	3	30.0
Rice straw	240	8	6	2	25.0
Rice straw, heat treated	797	11	11	0	0

1 week and all the polished rice fed was heat treated at a temperature of about 100° C. in a seed-house drying tower for at least 2 weeks. Similarly, any vitamin E in brewers' dried grains was inactivated by tripling the heating time in the drying drums at the brewery. The vitamin E found occasionally in lots of dry skim milk was destroyed by adding about 10 per cent of lard and allowing the mixture to stand at room temperature for several months or until the fat became rancid.

The chemical composition of each feed was determined by official A.O.A.C. methods (2). Calcium and phosphorus content was determined by the method developed by Morris *et al.* (19).

Cattle used. Fifteen calves of mixed breeding, consisting of nine females and six males, were in the original group. Two of these, E430, a male, and E438, a female, were used as positive control animals. During the progress of the experiment a few calves other than descendants of the original group were added. Two of these, E607 and E615, also were used as positive controls. A tocopherol supplement also was fed to E559 beginning about 3 weeks before her

TABLE 2
Data relating to the cattle used in the experiment

Herd no.	Breed	Birth date	Sex	Generation	Herd no. of		Age when removed from experiment	Remarks
					dam	sire		
						(Date) (Yr.) (Mo.)		
E348a	Gr. Hol.	6-14-39	F	P ₁	Unknown	Unknown	6-13-43 4	Discontinued. Died suddenly 6-29-43
E360	Gr. Hol.	5-11-40	F	"	"	"	5-10-45 5	Died suddenly
E383	Gr. Hol.	6-23-40	F	"	"	"	2-1-44 3	Died suddenly
E384	Gr. Hol.	7-1-40	F	"	"	"	4-10-43 2	Died suddenly
E390	Guern.	8-22-40	M	"	"	"	4-7-44 3	Died suddenly
E394	Guern.	12-25-40	M	"	"	"	3-8-43 2	Slaughtered
E396	Jersey	1-3-41	M	"	"	"	3-8-43 2	Slaughtered
E397	Jersey	2-6-41	M	"	"	"	2-3-44 3	Sold for slaughter
E398	Jersey	5-7-41	M	"	"	"	2-3-44 3	Slaughtered
E401	Gr. Hol.	8-14-41	F	"	"	"	3-15-43 1	Discontinued
E403	Gr. Jer.	9-22-41	F	"	"	"	1-28-44 2	Died suddenly
E405	Gr. Hol.	9-23-41	F	"	"	"	8-13-45 3-	Discontinued
E408	Gr. S. Horn	9-23-41	F	"	"	"	1-28-44 2	Discontinued
E413	Gr. Hol.	10-5-41	F	"	"	"	1-28-44 2	Discontinued
E430b	Guern.	12-24-41	M	"	"	"	4-21-44 2	Slaughtered
E438b	Gr. Hol.	2-12-42	F	"	"	"	3-21-45 3	Died suddenly
E480	Gr. Jer.	3-30-43	F	F ₁	E390	E397	8-22-45 2	Died suddenly
E481	Gr. Jer.	4-12-43	F	"	E380	"	6-26-45 2	Died suddenly
E482	Gr. Jer.	4-12-43	M	"	E383	"	10-15-45 2	Died suddenly
E490	Gr. Jer.	10-8-43	M	"	E405	E398	10-10-43 2 days	Died
E496	Gr. Jer.	10-30-43	F	"	E401	E397	3-8-44 0	Accidental death
E507	Gr. Jer.	1-6-44	F	"	E413	"	11-1-45 1	Died suddenly
E511	Gr. Jer.	1-17-44	F	"	E408	"	7-27-44 0	Broke leg—slaughtered
E516	Gr. Jer.	2-21-44	M	"	E403	"	8-21-45 1	Slaughtered
E541	Gr. Jer.	7-8-44	F	"	E380	"	4-4-46 1	Died suddenly
E558	Gr. Jer.	5-8-45	F	F ₁	E507	E482	7-8-47 2	Died suddenly
E559a	Gr. Jer.	5-9-45	F	F ₁	E380	"	3-15-49 3	Discontinued
E562	Gr. Jer.	6-23-45	F	F ₁	E481	"	6-28-45 0	Died—indigestion
E573	Gr. Jer.	11-27-45	F	F ₁	E541	"	10-19-47 1	Died suddenly
E585	Gr. Hol.	4-15-46	M	P ₁	Unknown	Unknown	12-18-47 1	Sold—difficult to handle
E595	Gr. Hol.	6-25-46	F	"	"	"	3-15-49 2	Discontinued
E607b	Gr. Hol.	7-14-46	F	"	"	"	3-15-49 2	Discontinued
E612	Gr. S. Horn	5-1-46	F	"	"	"	3-15-49 2	Discontinued
E615b	Gr. Hereford	8-12-46	F	"	"	"	3-15-49 2	Discontinued
E639	Gr. Jer.	10-17-47	M	F ₁	E573	E585	11-30-48 1	Sold for slaughter
E641	Gr. Jer.	10-24-47	F	F ₁	E559	"	11-30-48 1	Sold for slaughter

^a Placed on experiment 6-3-42.

^b Control animal fed synthetic alpha tocopherol once weekly at rate of 0.4 g./100 lb. weight.

^c Fed mixed tocopherols, 2 g. daily after 10-3-47.

first calving and until she was discontinued on experiment about 18 months later. Data relating to the cattle used in the experiment are presented in table 2.

Feeding and Management. The ration fed was planned to provide adequate amounts of all known essential nutrients excepting vitamin E. It was made up of some of the feedstuffs listed in table 1, along with a few others that earlier tests had shown to be relatively free of vitamin E. The ration consisted of rice straw as the sole roughage and a concentrate mixture which was designed to be palatable and to provide the nutrients needed. A mixture typical of those fed contained 25 per cent polished rice, 30 per cent brewers' dried grains, 18 per cent distillers' grains (solvent extracted), 11 per cent corn starch, 9 per cent dry skim milk, 4 per cent rendered lard, 2 per cent steamed bone meal and 1 per cent iodized salt. Delsterol (2000D) was added as a source of vitamin D, at the rate of 0.6 g. per lb. of concentrate mixture.

Steamed bone meal and iodized salt were available free choice to all animals. A vitamin A concentrate (potency approximately 8000 I.U.) was fed once daily to each animal at the rate of approximately 10,000 units (I.U.) per 100 lb. of weight (14). It was fed directly to calves, but for older animals, it was mixed with the grain at time of feeding. Table 3 presents the results of several vitamin E tests made on the rations fed.

TABLE 3
Vitamin E tests of various rations fed to cattle

Ration no.	Female rats					Male rats			
	No. of rats used	Av. amt. eaten by time mated	No. of complete resorptions	No. of live litters	Live litter efficiency	No. of rats used	Days fed test ration	Testes, % of normal weight	Av. stage of testicular degeneration*
		(g.)			(%)				
1	10	1135	10	0	0	10	100	55.8	4.35
2	9	979	9	0	0	10	100	59.4	4.00
3	9	984	9	0	0	10	100	69.5	3.25
4	7	987	7	0	0	10	100	61.6	4.10

* Stages of degeneration range from 0.0 to 5.0 for the most advanced stage.

All calves were fed whole milk until about 3 weeks old, followed by fresh skim milk to about 6 months of age. The whole milk fed to the calves born in the herd was produced by cows receiving vitamin E-poor rations. Rice straw was fed *ad libitum* along with enough concentrates to provide the protein and energy required according to Morrison (20).

The positive control animals, E430, E438, E607 and E615, were fed according to the same plan as all the others except that a vitamin E-rich supplement was added to their diet. For E430 and E438 this consisted of the addition, once weekly, of sufficient synthetic *alpha*-tocopherol, dissolved at 2 per cent level in partially hydrogenated vegetable oil (Crisco), to provide approximately 0.4 g. *alpha*-tocopherol per 100 lb. weight of animal. E438 was fed at approximately twice this level after she calved. For E607 and E615 the tocopherol concen-

trate¹⁰ was incorporated in the grain mixture in such amounts as to assure an intake of about 5 mg. per kg. of body weight per day. E559 was fed a concentrate¹⁰ providing about 2 g. of mixed tocopherols daily from 3 weeks before her first calving until discontinued on experiment about 18 months later.

Tests made on feces from cattle fed vitamin E-poor rations, as well as on those from similar animals fed normal rations, indicated that vitamin E is not synthesized within the digestive tract. Ten female rats that consumed dried feces in amounts equivalent to from 60 to 620 g. of fresh feces obtained from cattle fed vitamin E-poor rations all showed complete resorption of fetuses during pregnancy and 10 males reared from weaning to 100 days of age on a ration containing 50 per cent on dry matter basis of fresh feces showed complete degeneration of the germinal epithelium. Three of ten females consuming 73 to 700 g. of feces from cattle fed normal rations gave birth to normal litters.

The calcium and inorganic phosphate content of the blood of each animal was determined at monthly intervals for over 3 years during the years 1941 through 1944. These analyses showed all animals to be in a state of *luxus nutrition* as far as these elements were concerned; the inorganic phosphorus of the plasma seldom was below 6.0 mg. per cent and frequently exceeded 9.0 mg. per cent. Following 1944, blood analyses were made at less frequent intervals. Invariably all values were well within the normal range as indicated by various investigators (12, 15, 21).

While on experiment the cattle were kept isolated. They were turned outdoors for exercise in a vegetation-free lot whenever weather permitted. The exercise lot was divided into two parts permitting segregation of animals whenever desired. Calves were kept in individual pens in the barn until large enough to be stanchioned. Shavings and waste rice straw were used for bedding.

Reproduction. The cattle were observed daily, especially for manifestations indicating development and functioning of the organs of reproduction. Sexual development and behavior among the bulls was tested by permitting them to mingle with females showing estrus. Beginning when about 6 months old, all males receiving the vitamin E-poor ration invariably showed marked libido during such contact. Semen specimens obtained intermittently by use of artificial vagina and rectal massage of ampula were studied for normality and fertility. Spermatogenesis was not affected, as examination and tests showed that all ejaculates obtained were normal in sperm activity, morphology and longevity.

Studies of the sexual development and activity in females included frequent observations for both physical and psychological signs of estrus, as well as rectal examinations of the uterus and ovaries for evidence of ovulation. These showed that the estrus cycle with all its characteristic and continuous changes including ovulation occurred regularly and in a normal manner starting when the heifers were 7 to 9 months old. Females exhibiting estrus invariably showed a strong desire to mount or ride other animals in the herd.

Breeding and calving records were kept for all animals. A summary of those

¹⁰ "Myvadry" prepared by Distillation Products, Inc., Rochester, N. Y.

relating to the females is presented in table 4. The data show that the reproducing ability of the cattle was not affected adversely by feeding the vitamin E-poor ration continuously through three generations.

The data in table 5 show that a total of only 30 services was required to produce 25 pregnancies in the 19 females of breeding age that were fed the vitamin

TABLE 4
Data relating to the breeding ability of cows

Herd no.	Cows					Calves				
	Times bred	No. of calving	Calving date	Age at calving		Length of gestation	Herd no.	Sex	Birth weight	Physical condition
				(yr.)	(mo.)					
									(lb.)	
E348 ^a	1	1	6-10-43.	3	3	275	E483	F	69	Very good
E380	1	1	4-12-43	2	11	279	E481	F	75	Very good
	2	2	7- 8-44	4	2	278	E541	F	77	Very good
	1	3	5- 9-45	5	0	277	E559	F	75	Very good
		Cow died	5-10-45.							
E383	1	1	4-12-43	2	10	283	E482	M	97	Excellent
	2		Cow died 2- 2-44.	Pregnant 186 days.						
E384	2		Cow died 4-10-43.	Pregnant 246 days.						
E390	1	1	3-30-43	2	7	281	E480	F	95	Excellent
	1		Cow died 4- 7-44.	Pregnant 240 days.						
E401 ^b	1	1	10-30-43	2	3	279	E496	F	85	Excellent
E403	1	1	2-21-44	2	5	284	E516	M	67	Excellent
	1		Cow died 8-13-45.	Pregnant 248 days.						
E405 ^b	1	1	10- 8-43	2	1	281	E490	M	78	Good
E408 ^b	3	1	1-17-44	2	4	285	F511	F	74	Excellent
E413 ^b	1	1	1- 6-44	2	3	270	E507	F	55	Good
E438 ^c	1	1	3-23-44	2	1	279	E522	F	72	Good
	2		Cow slaughtered 3-21-45.	Pregnant 180 days.						
F480	1	1	10-30-44	1	7	286	none	F		calf smothered
	1		Cow died 8-22-45.	Pregnant 225 days.						at birth
E481	1	1	6-23-45	2	2	279	E562	F	47	Fair
F507	1	1	5- 8-45	1	4	279	E558	F	55	Good
			Cow died 10-31-45							
E541	1	1	11-27-45	1	5	281	E573	F	78	Very good
			Cow died 4- 4-46							
F558	1		Cow died 7- 8-47.	Pregnant 245 days.						
E559 ^d	1	1	10-24-47	2	5	286	E641	F	80	Very good
	2 ^e	2	2-12-49			277	E682	F	80	Very good
F573	1	1	10-17-47	1	11	285	E639	M	61	Good
			Cow died 10-19-47							
E595	1	1	7-25-48	2	1	275	E672	M	87	Good
F607 ^c	1	1	9- 5-48	2	2	279	E674	F	72	Good
E612 ^e	1	1	2-26-49	2	7	276	E683	M	96	Good
E615 ^e	1	1	8-11-48	2	0	280	E673	M	80	Good

^a Started on experiment 6-3-42 and removed from experiment 6-12-43.

^b Removed from experiment 1-28-44.

^c Positive control animal.

^d Fed 2 g. mixed tocopherols daily after 10-3-47.

^e Bred to bull fed normal ration.

E-poor rations. An average of only 1.2 services were required per conception. The 6 bulls used had an average breeding efficiency of 83.3 per cent. All F₁ and F₂ generation heifers conceived on the first service. All of the positive control heifers also conceived on the first service for their first calving, but E438 and E559 each required two services for the second pregnancy.

All heifers dropped their first calf when about 2 years old. Two F_1 generation heifers were less than 18 months old when they calved. One F_1 generation bull mated successfully when only about 10 months old, thus indicating that the vitamin E-deficient ration did not delay sexual maturity. One cow (E380) gave birth to three calves within a period of 25 months, with only 10 months between the last two parturitions. There were no abortions during the experiment. The length of all gestation periods were within the normal range (17). All calves were normal in size and vigor at birth. Fetal membranes invariably were expelled within several hours after calving occurred. No abnormalities were found in the reproductive organs of animals that died or were slaughtered.

Growth and physical condition. All animals were weighed at birth or when placed on experiment, and subsequently at 30-day intervals. Weights of experimental animals at various ages and the normal weights (25) at comparable ages of cattle of the several breeds represented are presented in table 6. These com-

TABLE 5

Breeding ability of bulls fed vitamin E-deficient rations as indicated by number of services required per conception when mated to cows fed similar rations

Bull	Services	Conceptions	Services per conception	Breeding efficiency
	(no.)	(no.)	(av.)	(%)
E394	1	1	1.00	100.0
E397	16	11	1.45	68.8
E398	1	1	1.00	100.0
E482	7	7	1.00	100.0
E585	2	2	1.00	100.0
Pure bred Guernsey*	3	3	1.00	100.0
Total or av.	30	25	1.20	83.3

* Temporarily fed vitamin E-deficient ration.

parisons, although not entirely satisfactory because of the mixed inheritance of the experimental animals, show that some of the cattle in the original group were below normal weight for their breed but that their descendants invariably were considerably above it.

The cattle displayed few abnormalities in action or appearance. The hocks of one F_2 generation heifer (E558) were swollen slightly at birth, a condition which persisted throughout her entire life. The swellings remained soft and were most prominent anteriorly, extending several inches above and below the joint.

All animals, after several months on the experimental diet, invariably exhibited some degree of "tongue lolling," a phenomenon not infrequently observed in cattle fed rations deficient in some essential factor or factors. A marked desire to lick fence or stall boards also was evinced. Feeding of a mineral mixture containing cobalt, manganese, copper, iron and magnesium for several months had no visible effect in alleviating the symptoms noted above or in improving the physical condition of the cattle.

Death losses. Thirteen out of the 28 animals fed the vitamin E-poor rations

for 1 year or more died suddenly at ages ranging from 21 months to 5 years. The daughter of E480 smothered at birth. Two others, E490 and E562, died soon after they were born, due to improper care by an inexperienced caretaker. E496 died as the result of an accident and E511 was slaughtered after she broke a leg falling on slippery pavement.

TABLE 6

Weight in lb. of cattle at birth and at various intervals up to 720 days of age with breed normal^a weights

Herd no.	Breed	Sex	Age (d.)						
			Birth	30	180	360	540	720	
E380	Grade Holstein	F	71	76	234	507	732	960	
E383	Grade Holstein	F	90	105	328	578	815	1,022	
E384	Grade Holstein	F	70	77	223	425	700	903	
E390	Grade Holstein	F	88	90	316	532	840	1,014	
E401	Grade Holstein	F	84	94	314	585	837	1,079	
E405	Grade Holstein	F	85	99	309	544	773	973	
E408	Grade Short Horn	F	75	113	301	564	845	1,049	
E413	Grade Holstein	F	82	97	334	504	726	1,010	
E438 ^b	Grade Holstein	F	75	99	257	589	828	1,022	
E595	Grade Holstein	F	95	133	345	580	725	990	
E607 ^b	Grade Holstein	F	84	135	325	536	740	966	
E612	Grade Short Horn	F	95	131	335	481	705	935	
Normal weight, Holstein			F	90	112	355	632	845	1,069
E403	Grade Jersey Short Horn	F	50	61	205	403	616	781	
E480	Grade Jersey	F	95	106	347	628	892	851	
E481	Grade Jersey	F	75	88	262	541	766	1,003	
E507	Grade Jersey	F	60	91	383	629	746		
E511	Grade Jersey	F	74	104	354				
E558	Grade Jersey	F	55	88	290	499	694	874	
E559	Grade Jersey	F	75	113	354	723	916	1,066	
E573	Grade Jersey	F	78	101	337	594	803	884 ^c	
E615 ^b	Grade Hereford	F	68	83	298	483	705	865	
E641	Grade Jersey	F	79	117	309	568			
Normal weight, Jersey			F	53	67	243	450	601	733
E585	Grade Holstein	M	83	125	390	781	1,190		
Normal weight, Holstein			M	94	125	399	741	1,176	1,438
E396	Jersey	M	43		264	588	824	1,027	
E397	Jersey	M	62		282	558	744	1,022	
E398	Jersey	M	64	82	281	573	806	970 ^d	
E482	Grade Jersey	M	97	127	414	756	1,011	1,258	
E516	Grade Jersey	M	67	95	342	708			
E639	Grade Jersey	M	62	77	267	545			
Normal weight, Jersey			M	60	78	282	531	745	969
E394	Guernsey	M	58	78	240	546	765	945	
E430 ^b	Guernsey	M	76	81	261	543	799	1,039	
Normal weight, Guernsey			M	71	87	291	609		

^a Mo. Agr. Expt. Sta. Bull. 336. 1934.

^b Control animal.

^c Weight at 707 d. of age.

^d Weight at 662 d. of age.

Unlike any of the other animals, E348 was fed the vitamin E-poor ration for only about 1 year starting when she was about 2 years old. She was bred and conceived on the first service to E394 about 3 months after she was started on the experiment. The gestation period which followed was uneventful and she appeared to be in excellent physical condition at time of parturition. Two days after giving birth to a normal heifer calf she was discontinued on experiment and placed on a normal ration consisting of alfalfa hay, corn silage and ground grains. During the first few days after changing to the new ration her feed consumption was normal, but after that she refused all feed except an occasional sip of water. She died on the 19th day after calving. The post-mortem examination failed to reveal the cause of her death. It is possible that the extreme change in ration upset the established microflora in the digestive tract to such an extent as to prove fatal to the cow.

The other 12 animals which died suddenly displayed few premonitory symptoms of their impending death. Several collapsed while standing in their stanchions consuming their rations. Most of them showed some loss of appetite for a month or more before their death, with loss in weight or decrease in rate of gain. One heifer (E558) developed a condition of profuse salivation with almost complete anorexia several weeks before her death. Several days before her death, she became too weak to stand. The only male which died suddenly was E482. He was being used as herd sire at the time of his death and was about 30 months old. His weight declined from a maximum of 1,344 lb. on June 16 to 1,275 lb. at the time of his death 4 months later. He had the habit of scattering his feed making it difficult to obtain an accurate record of the amount consumed.

In no case did gross post mortem examinations reveal pathologic changes sufficiently severe to indicate the specific cause of death. Slight hemorrhages were found in the brain of some of the cattle and in others they were apparent on the bowels and occasionally on the heart and pancreas. These sudden deaths, as was shown by Gullickson and Calverley (9), probably resulted from cardiac injury induced by restricting them to a ration deficient in vitamin E. This phase of the study will be considered more fully in a later report.

Milk and fat production. Records were kept of the milk and fat production of each cow. The milk produced at each milking of every cow was weighed and on one day each month, the fat content of a composite of the milk of each cow was determined by the Babcock method.

Of the cows fed vitamin E-poor rations, only E380 completed two lactation periods and only two others completed one. Three cows died and another was discontinued on experiment before completing the first lactation period. The control animal E438 was slaughtered for the purpose of obtaining body fat for chemical and biological tests after she had milked 388 days. The other control animals, E607 and E615, were discontinued after milking about 7 months. The cow E559, which was fed a tocopherol supplement from 3 weeks before her first calving and until discontinued on experiment about 18 months later, experienced two normal calvings and completed one lactation period of 383 days. The milk and fat production of each animal is reported in table 7.

TABLE 7
Milk and butterfat production of cows on experiment

Herd no.	Age at calving		Milking period	Milk		Fat
	(yr.)	(mo.)	(d.)	(lb.)	(%)	(lb.)
E380	2	11	277	6904.1	3.04	209.9
	4	2	232	6764.1	3.20	216.5
E383 ^a	2	10	289	8186.9	2.93	239.9
E390	2	7	317	10135.3	2.85	288.9
E403	2	5	447	9028.8	3.25	293.4
E438 ^b	2	1	358	9078.9	3.31	300.5
E480 ^a	1	7	290	7868.2	3.92	308.4
E507 ^a	1	4	171	3332.6	3.94	131.3
E559 ^a	2	5	383	6764.3	3.38	228.6
E595	2	1	234 ^d	4714.7	3.14	148.0
E607 ^b	2	2	191 ^d	4186.9	2.52	105.5
E615 ^b	2	0	216 ^d	3028.1	3.11	94.2

^a Died suddenly before end of lactation period.

^b Positive control animal.

^c Fed mixed tocopherols, 2 g. daily during entire lactation period.

^d Experiment discontinued before completing lactation period.

DISCUSSION

Although the feeding of vitamin E-poor rations apparently did not affect the ability of cattle to reproduce, it nevertheless is significant that out of the dozen or more animals which died suddenly from the characteristic heart ailment described by Gullickson and Calverley (9), six had been pregnant 6 to 8 months at the time death occurred, three died within 3 days and one (E348) 19 days after calving. However, pregnancy and parturition probably were only indirectly the cause of these deaths. A critical vitamin E shortage would be expected to develop during the last several months of the gestation period when the requirement for various essential nutritive factors has been shown to increase markedly (6). In rats during the corresponding period degenerative changes occur, not in the deficient mother but in the developing embryo, which eventually succumbs and is resorbed (8). Careful studies by Mason and Bryan (18) have shown that placental transmission of vitamin E in the rat is very small. It is possible that in the bovine it may be greater, resulting in sacrifice of the mother instead of the developing fetus. Whiting and Loosli (26), in experiments with sheep, goats and swine, found that adding tocopherols to the prepartum ration produced a highly significant increase in the tocopherol content of the blood plasma of the lambs and kids but no increase was observed in the pigs. Palmer *et al.* (23) have shown that deficiencies of protein and various minerals affect the cow more seriously than her unborn calf. The increased burden imposed during the stress of calving and initiation of lactation are other factors that would be expected to affect the already injured heart and the welfare of the cow during this period. Brody *et al.* (4) found that gestation increases heat production in cattle about 40 per cent above the non-gestating level during the last one-third of the gestation period, and cows lactating heavily produce about twice as much heat under normal feeding conditions as when not milking. Pulse

rate, respiration rate and ventilation rate were found to parallel the course of heat production.

It is possible that if the rations fed had been completely vitamin E-free, few if any of the females involved would have survived their first gestation period. That neither pregnancy nor lactation is essential to the development of the lethal condition, however, is suggested by the deaths of bull E482 and several of the non-pregnant females.

There is no indication that the vitamin E-deficient ration fed retarded the growth of cattle except insofar as it appeared to reduce feed consumption in a few cases. Neither did rate of milk and butterfat production appear to be affected adversely, for, as is indicated in table 7, the quantity and fat content of the milk produced by the control cows, including E559, are not markedly different from that of cows fed vitamin E-poor rations. Recently it was shown by several workers (10, 24, 27) that feeding of mixed tocopherols to cows on normal rations has little or no effect on the quantity and fat content of milk produced.

SUMMARY AND CONCLUSIONS

A study was made to determine the role of vitamin E in the nutrition of cattle and especially as it relates to reproduction. A total of 30 animals of mixed breeding (8 males and 22 females), including those in the original group and their second, third and fourth generation descendants, were fed throughout their lives on rations providing adequate amounts of all nutritive factors known to be essential except vitamin E. The rations fed were incapable of supporting reproduction in rats when fed in liberal amounts. Four positive control animals, one bull and three cows, were fed exactly like the others except that each of them was fed a supplement providing either *alpha*-tocopherol or mixed tocopherols. Organs of reproduction developed normally in animals of both sexes. Spermatogenesis in males was not interfered with and all ejaculates studied were normal as to volume, sperm activity, morphology and longevity. The estrus cycle in its various phases, including ovulation, occurred regularly starting when heifers were about 7 months old.

A total of 30 services produced 25 conceptions in the cattle fed vitamin E-poor rations, but only 19 of these terminated in normal parturitions, since the other six cows died suddenly 1 to 3 months before they were due to calve. All calves born were normal in size and vigor at birth. There were no abortions and fetal membranes invariably were expelled within several hours after the calves were born. The average breeding efficiency of the five bulls used was 83.3 per cent.

One bull about 30 months old and 12 females ranging in age from 21 months to 5 years died suddenly, apparently from cardiac failure. Six of the females had been pregnant 6 to 8 months when death occurred and three died within 3 days and one 19 days after calving. Gross post mortem examinations failed to reveal pathologic changes sufficiently marked in any of them to indicate a specific cause of death.

Vitamin E does not appear to be required by cattle for successful reproduction, but long-continued feeding of rations containing very much less vitamin E than is present normally under practical conditions is likely to prove fatal to such animals. Feeding rations in practice as deficient as those fed in this experiment is exceedingly unlikely as all feeds commonly fed to cattle are relatively rich in vitamin E.

REFERENCES

- (1) ASDELL, S. A. Nutrition and the Treatment of Sterility in Dairy Cattle: A Review. *J. Dairy Sci.*, **32**: 60-70. 1949.
- (2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 5th ed. 1940.
- (3) BAY, F., AND VOGT-MOLLER, P. Fortsatte Undersøgelser Angaaende Sterilitetsbehandling af Koer og Grisesoer med Hvedekimolie. *Medlemsbl. Danske Dyrlaeger Forening*, **17**: 166-169. 1934.
- (4) BRODY, S., WORSTELL, D. M., RAGSDALE, A. C., AND KIBLER, H. H. The Effect of Lactation and Gestation on Heat Production and Cardiorespiratory Activities of Dairy Cattle and Rats. (Abs.) *J. Dairy Sci.*, **31**: 666. 1948.
- (5) CARELL, C. A., AND ELLIS, N. R. The Vitamin E Content of Certain Varieties of Wheat, Corn, Grasses and Legumes as Determined by Rat Assay. *J. Nutrition*, **23**: 625-632. 1942.
- (6) ECKLES, C. H., PALMER, L. S., GULLICKSON, T. W., FITCH, C. P., BOYD, W. L., BISHOP, LUCILLE, AND NELSON, J. W. Effects of Uncomplicated Phosphorus Deficiency on Estrous Cycle, Reproduction and Composition of Tissues of Mature Dairy Cows. *Cornell Vet.*, **25**: 22-43. 1935.
- (7) EVANS, H. M., AND BISHOP, K. S. On the Existence of a Hitherto Unrecognized Dietary Factor Essential for Reproduction. *Science*, **56**: 650-651. 1922.
- (8) EVANS, H. M., AND BUER, G. O. The Antisterility Vitamin Fat Soluble E. *Mem. Univ. Calif.*, **8**. 1927.
- (9) GULLICKSON, T. W., AND CALVERLEY, C. E. Cardiac Failure in Cattle on Vitamin E-free Rations as Revealed by Electrocardiograms. *Science*, **104**: 312-313. 1946.
- (10) GULLICKSON, T. W., FITCH, J. B., AND GILMORE, L. O. Effect of Feeding Tocopherols to Dairy Cows on the Quantity and the Fat Content of Milk Produced. *J. Dairy Sci.*, **31**: 557-560. 1948.
- (11) GUNN, WALLACE R. Some Observations on the Effects of the Use of Wheat Germ Oil in a Herd of Purebred Holstein Cattle. *Brit. Columbia Dept. Agr. Livestock Circ.* **32**: 1-4. 1941.
- (12) HAAG, J. R., AND JONES, I. R. The Calcium and Inorganic Phosphorus Content of the Blood Plasma of Normal Dairy Cattle. *J. Biol. Chem.*, **110**: 439-441. 1935.
- (13) HARRIS, P. L., SWANSON, W. J., AND HICKMAN, K. C. D. Covitamin Studies. VI. Effect of Tocopherol Supplementation on the Output of Vitamin A, Carotene and Fat by Dairy Cows. *J. Nutrition*, **33**: 411-428. 1947.
- (14) HAET, G. H. Vitamin A Deficiency and Requirements of Farm Mammals. *Nutrition Abstracts & Revs.* **10**: 261-272. 1941.
- (15) HENDERSON, H. O., AND WEAKLEY, C. E. The Effect of Feeding Different Amounts of Calcium and Phosphorus upon the Growth and Development of Dairy Animals. *West Va. Agr. Expt. Sta. Bull.* **231**. 1930.
- (16) JONES, I. R., AND EWALT, H. P. A Preliminary Report on Vitamin E in the Rations of Dairy Cows. Paper delivered at Western Div., Am. Dairy Sci. Assoc. meeting. Pp. 1-6. 1936.
- (17) KNOOP, C. E., AND HAYDEN, C. C. A Study of the Length of Gestation and Service Record of Dairy Cows. *Ohio Agr. Expt. Sta. Bimonthly Bull.* **19**, 166: 8-13. 1934.

- (18) MASON, K. E., AND BRYAN, W. L. Placental and Mammary Transfer of Vitamin E in the Rat. *J. Nutrition*, 20: 501-517. 1940.
- (19) MORRIS, H. P., NELSON, J. W., AND PALMER, L. S. Quantitative Determination of Calcium, Magnesium and Phosphorus in Feeding Stuffs and Cattle Excreta. *Ind. Eng. Chem., Anal. Ed.*, 3: 164-167. 1931.
- (20) MORRISON, F. B. Feeds and Feeding. 20th ed., unabridged. 1050 pp. Morrison Publishing Co., Ithaca, N. Y. 1936.
- (21) PALMER, L. S., CUNNINGHAM, W. S., AND ECKLES, C. H. Normal Variations in the Inorganic Phosphorus of the Blood of Dairy Cattle. *J. Dairy Sci.*, 13: 174-195. 1930.
- (22) PALMER, L. S. Biological Assay of Vitamin E. Application to Wheat Germ and Wheat Germ Oil. *Ind. Eng. Chem., Anal. Ed.*, 9: 427-429. 1937.
- (23) PALMER, L. S., GULLICKSON, T. W., BOYD, W. L., FITCH, C. P., AND NELSON, J. W. The Effect of Rations Deficient in Phosphorus and Protein on Ovulation, Estrons and Reproduction of Dairy Heifers. *J. Dairy Sci.*, 24: 199-210. 1941.
- (24) PHILLIPS, P. H., KASTELIC, J., AND HART, E. B. The Effect of Mixed Tocopherols on Milk and Butterfat Production of the Dairy Cow. *J. Nutrition*, 36: 695-702. 1948.
- (25) BAGSDALE, A. C. Growth Standards for Dairy Cattle. *Mo. Agr. Expt. Sta. Bull.* 336. 1934.
- (26) WHITING, F., AND LOOSLI, J. K. The Placental and Mammary Transfer of Tocopherols (Vitamin E) in Sheep, Goats and Swine. *J. Nutrition*, 36: 721-726. 1948.
- (27) WHITING, F., LOOSLI, J. K., KRUKOVSKY, V. N., AND TURK, K. L. The Influence of Tocopherols and Cod-Liver Oil on Milk and Fat Production. *J. Dairy Sci.*, 32: 133-138. 1949.
- (28) WILLMAN, J. P., LOOSLI, J. K., ASDELL, S. A., MORRISON, F. B., AND OLAFSON, P. The Prevention and Cure of Muscular Stiffness ("Stiff-lamb" Disease) in Lambs. *J. Animal Sci.*, 4: 128-132. 1945.

THE RATE OF PHOSPHATASE INACTIVATION IN MILK¹

S. A. LEAR AND H. G. FOSTER^{2, 3}

Department of Dairy Industry, Rutgers University, New Jersey

The phosphatase test over a period of years has proved to be an excellent method of determining whether holder-processed milk is pasteurized properly. Its wide acceptance by industry and health authorities is based on research and the ease and speed with which the results are obtained. In England, where Kay and Graham (10) first introduced the test in 1933, milk must be heated to and held at 145° F for 30 minutes to be considered pasteurized. In addition, the enzyme must be inactivated completely. In areas where the pasteurization temperature may be 142 or 143° F with a 30-minute holding period and a partial inactivation of the enzyme is acceptable, the interpretation of the results may vary considerably. Evidence of this is noted readily in the several modifications of the phosphatase test and standards which are recognized and used (1).

Kay and Graham (10, 11) and other workers (5, 7, 8, 9, 15, 16) have recognized that the time required to heat the milk to pasteurization temperature may affect the results of the test. This is especially true when higher temperatures of pasteurization are used, as in the flash process. The inactivation effect of the preheating time on flash-pasteurized milk may be great enough to indicate proper pasteurization, even though the milk was not held at the required temperatures for the necessary time. With the exception of a recently published study (8) no data were found in the literature indicating the exclusive effect of preheating time. Herschdorfer (6) published experimental data to show that an inverse relationship exists between the rate of cooling and the rate of phosphatase inactivation in milk.

The purpose of this study is to determine the rate of phosphatase inactivation in milk with a minimum influence of either preheating or cooling time.

REVIEW OF LITERATURE

Much interest has developed in the type of reaction involved when phosphatase is inactivated by heat in milk. Unfortunately, whether heat inactivates phosphatase in milk at a monomolecular rate has not been established definitely to everyone's satisfaction. Sanders and Sager (14, 15) have reported that a straight line results when the logarithms of the times of heating are plotted against the corresponding temperatures. Hetrick and Tracy (7) reported that semilogarithmic relationship between temperature and time over the temperature range of 143 to

Received for publication December 7, 1948.

¹ Journal series paper of the New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Dairy Industry.

² Submitted by H. G. Foster in partial fulfillment of the requirements for the M.S. degree at Rutgers University.

³ Presented at the meetings of American Dairy Science Association, Eastern States Division, Springfield, Mass., September 20, 1948.

185° F. was found to exist for the thermal destruction of phosphatase. On the other hand, Holland and Dahlberg (9) show a curve which appears to be a straight line until temperatures around 170° F. are attained, and then a hook may be observed. Basic information on this subject is imperative and could be used to predict the effect of a higher temperature on the inactivation of phosphatase as compared to a lower temperature if a semilogarithmic relationship between temperature and time were established and expressed in units independent of the pasteurization process and phosphatase test employed.

METHODS

Fresh whole milk from several different sources was used. Samples (approximately 20 ml.) were sucked through a copper coil into an attached U tube by means of a pump. The copper tubing was 25 feet long and had an inside diameter of 0.125 inches. The coil and U tube were maintained in a thermostatically-controlled water bath at a predetermined temperature within a range of $\pm 0.1^\circ$ C. A thermocouple was used to determine the temperature of the milk as it entered the glass tube and during the holding period. It required 7 seconds (± 1 second) for a unit of milk to travel through the coil. Samples were pipetted into test tubes held in ice water at the end of a holding period which was accurate to within ± 3 seconds. The (Rapid) Laboratory Phosphatase Test developed by the New York City Health Department (1) was used, together with a Pfaltz and Bauer fluorophotometer, Model B, with a 660-m μ filter, to determine the degree of phosphatase inactivation. Photometer units were converted to parts per million of phenol by reference to a prepared standard phenol curve (12). Time-temperature conditions that produced color equal to 0.5 p.p.m. of phenol were selected as end points because the data were reproducible under these circumstances. Ball's method (2) was used to interpret the data and to compare them with the results of other workers (4, 7, 8, 14).

EXPERIMENTAL

Fresh samples of milk were kept at approximately 5° C. until poured into a test tube (15 \times 150 mm.) which was attached immediately to the copper coil. The laboratory equipment is shown in figure 1.

Because of the relatively large capacity of the vacuum pump, milk flowed from the test tube and through the coil instantly after the pump was started and the system was closed. It always was necessary to maintain samples for varying periods at a predetermined temperature to determine the holding time required to inactivate the enzyme to the desired degree. After this point was located samples in duplicate were held for those times necessary to inactivate phosphatase to the desired degree and slightly under and over this point. In this manner the end-point of 0.5 p.p.m. (± 0.4) of phenol was checked not only by duplicate samples but also by its relative position in a series of samples for each degree of temperature from 60 to 71° C., inclusive. The results of this study are shown in table 1. These data were used to locate the curve in figure 2. The curve indicates that the rate of phosphatase inactivation in milk is a mono-

molecular reaction, at least from a practical view point, over the range of temperature considered. Ball's mathematical technic was used to interpret the data. In this method, experimental data are presented in graph form on semi-logarithmic paper. The vertical scale represents the logarithm of the time in minutes and the abscissa represents degrees of temperature. The standard symbol for designating the slope value of the curve is Z , expressed as degrees of temperature obtained from one logarithmic cycle on semi-logarithmic paper. The Z value for the curve in figure 2 is 4.9°C . When Ball's method was used to interpret the published data of several workers (4, 7, 9, 14), Z values of 4.60, 4.95, 4.50, and 4.85°C ., respectively, were obtained.

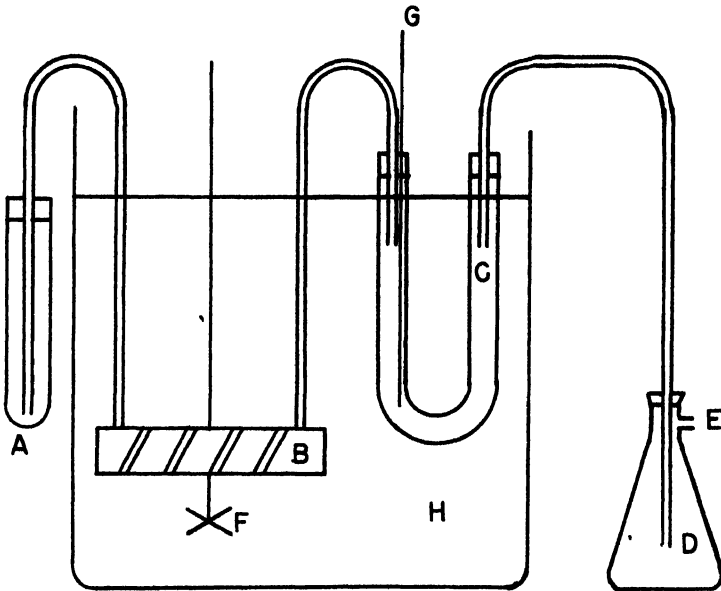


FIG. 1. Laboratory Pasteurizer. A- Test tube containing milk sample. B- Pasteurizer coil, 25 ft. long, 0.125 in. internal diameter (copper). C- U Tube. D- Trap in suction line. E- Line to suction pump. F- Electric water agitator. G- Thermocouple, iron-constantan. H- Constant temperature water bath.

DISCUSSION

It is difficult to compare much of the published information about the heat inactivation of phosphatase because experimental methods and units used to express the results are different in many of the reported studies. Several workers (3, 4, 8, 9, 11, 13, 15, 16) have recognized these variables.

After the present study was started data were reported (7, 14) to indicate that the rate of phosphatase inactivation was a straight line reaction over the temperature range usually employed for pasteurizing milk when the preheating and cooling times were extremely short. Calculated Z values, based on these data, were found to be 4.95°C . and 4.85°C ., respectively. Our own data indicate

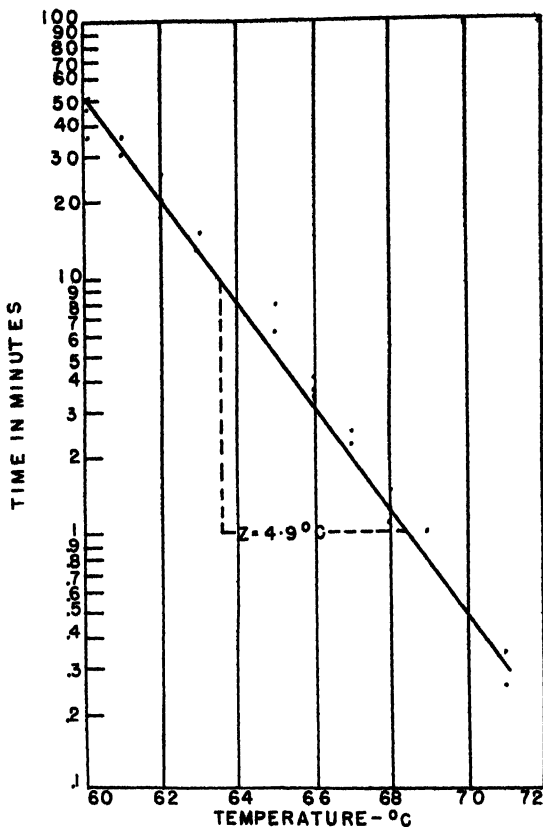


FIG. 2. The rate of phosphatase inactivation in milk.

a Z value of 4.9°C . The high degree of agreement of the Z values based on data from three different laboratories indicates that this method of expressing the effect of heat on phosphatase may be of practical value. When other data (4, 9) were used, the Z values were found to be 4.6°C . and 4.5°C ., respectively. These figures show the effect of preheating time on the slope of the curve.

TABLE 1

Effect of time and temperature on the inactivation of phosphatase in milk

Temperature	Holding time	Phenol ^a	Temperature	Holding time	Phenol ^a
(°C.)	(Min.)	(p.p.m.)	(°C.)	(Min.)	(p.p.m.)
60	40	0.35	66	3.50	0.84
61	35	0.17	67	2.50	0.53
62	21	0.33	68	1.25	0.39
63	15	0.47	69	1.00	0.78
64	7	0.64	70	0.50	0.55
65	7	0.54	71	0.30	0.30

^a Av. of at least three samples, each run in duplicate.

The significance of the variations in Z values for two or more curves with a common point is that the lowest one implies the highest relative inactivation rate for a high temperature as compared to that of a low temperature. It should be emphasized that the Z value is an expression of the inactivation rate and is not to be misconstrued as an intensity factor or lethal value. Equal Z values indicate that the reactions proceed at the same rate; that is, the curves are parallel. However, two phenomena may have the same Z value but one may take a more intense heat treatment than the other to accomplish destruction. This is illustrated in the data presented by Sanders and Sager (15).

It is impossible to say what difference between slope values is commercially significant in a milk plant at present. The time-temperature curve of phosphatase inactivation under commercial conditions will be influenced by the preheating and cooling times and will have a slope value characteristic for the conditions under which the inactivation is accomplished. These effects are not only applicable to the enzyme phosphatase but also to bacteria. Commercial experience shows that the effect of holder- and flash-pasteurization conditions may be practically equal for destroying pathogens ordinarily found in milk but unequal for thermoduric bacteria. This means that the Z values of the thermal death time curves for pathogens are lower than those for thermoduric bacteria.

CONCLUSIONS

1. Ball's methods (2) may be used as a basis for comparing the effect of heat on the rate of phosphatase inactivation in milk pasteurized by different methods.
2. The Z value for the rate of phosphatase inactivation in milk in this study is 4.9°C .

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Dr. David Levowitz of the New Jersey Dairy Laboratories for his advice during the course of this study.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 8th ed. New York, N Y. 1941.
- (2) BALL, C. O. Short Time Pasteurization of Milk. *Ind. Eng. Chem.*, 35: 71-84. 1943.
- (3) BURGWARD, L. H. The Phosphatase Test. A Review of the Literature on its Application for Detecting Irregularities in the Pasteurization of Milk and Dairy Products. *J. Dairy Sci.*, 22: 853-873. 1939.
- (4) BURGWARD, L. H., AND GIBERSON, E. M. An Evaluation of the Various Procedures for Making Phosphatase Tests. *J. Milk Technol.*, 1: 11-24. 1938.
- (5) GILCREAS, F. W. Value of Phosphatase as an Indicator of Pasteurization. *Am. J. Pub. Health*, 29: 158-162. 1939.
- (6) HERSCHDORFER, S. Inactivation of Phosphatase. *Enzymologia*, 1: 96. 1936.
- (7) HETRICK, J. H., AND TRACY, P. H. A Solution for Time and Temperature Relationships for Inactivating the Phosphatase Enzyme in Milk. *J. Dairy Sci.*, 31: 706. 1948.
- (8) HETRICK, J. H., AND TRACY, P. H. Effect of High-Temperature Short-Time Heat Treatments on Some Properties of Milk. I. Inactivation of the Phosphatase Enzyme. *J. Dairy Sci.*, 31: 867-879. 1948.

- (9) HOLLAND, R. F., AND DAHLBERG, A. C. The Effect of the Time and Temperature of Pasteurization upon Some of the Properties and Constituents of milk. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 254. 1940.
- (10) KAY, H. D., AND GRAHAM, W. R., JR. Phosphorus Compounds of Milk. VI. The Effect of Heat on Milk Phosphatase. J. Dairy Research, 5: 63-74. 1934.
- (11) KAY, H. D., AND GRAHAM, W. R., JR. The Phosphatase Test for Pasteurized Milk. J. Dairy Research, 6: 191-203. 1935.
- (12) Pharmacopoeia of U.S., XII. pp. 357-358. 1942.
- (13) SANDERS, G. P., AND SAGER, O. S. Modification of the Phosphatase Test as Applied to Cheddar Cheese and Application of the Test to Fluid Milk. J. Dairy Sci., 29: 737-749. 1946.
- (14) SANDERS, G. P., AND SAGER, O. S. Present Status of the Phosphatase Test. J. Milk Food Technol., 11: 67-75. 1948.
- (15) SANDERS, G. P., AND SAGER, O. S. Heat Inactivation of Milk Phosphatase in Dairy Products. J. Dairy Sci., 31: 845-857. 1948.
- (16) SCHAREE, H. A Rapid Phosphomonoesterase Test for Control of Dairy Pasteurization. J. Dairy Sci., 21: 21-34. 1938.

A STUDY OF THE DIACETYL IN CHEESE. I. DIACETYL CONTENT AND FLAVOR OF CHEDDAR CHEESE¹

HAROLD E. CALBERT AND WALTER V. PRICE

Department of Dairy Industry, University of Wisconsin

Diacetyl is a prominent element in the flavor of many food products. This is true particularly of food products that are produced by a fermentation process.

Prill *et al.* (9) have discussed the various properties of diacetyl as well as its higher homologues. In their work the general chemical structure of the *alpha*-dicarbonyl compounds was presented with particular reference to the contribution of diacetyl and its homologues to butter flavor and aroma. While the influence of the diacetyl content on the flavor and aroma of butter and starter cultures has been investigated to a considerable extent, little work has been done to determine the diacetyl content of cheese. Both Crocker (2) and Davies *et al.* (6) have indicated that diacetyl could be a possible constituent of cheese aroma. In listing the various flavors of cheese, Davis (7) referred to the buttery aroma and suggested that the substance responsible was diacetyl. Davies *et al.* (6) attempted to determine the effect of adding diacetyl to the curd in making cheese. They reported that when diacetyl was added in the concentration of 1.7 p.p.m. to the curd, a cheese was produced that had a stinging effect on the tongue.

In 1941, Csiszár and Bákó (3) and Csiszár *et al.* (4, 5) conducted studies in Hungary on the diacetyl content of such types as Emmenthal, Ovári, Trappist, Edam, Roquefort, Camembert and Romadour. These investigators reported that the aroma and quality in cheese were better when the cheese was made with a culture which was highly active in acetylmethylcarbinol-diacetyl formation. The diacetyl content of the various types of cheese as reported by Csiszár *et al.* (4) ranged from none to as much as 1 mg. per 100 g. of cheese. This study was undertaken with these objectives: (a) to determine whether diacetyl is a component of cheese; (b) if diacetyl is present, to determine to what extent; and (c) to determine whether there is a correlation between cheese flavor and the amount of diacetyl present in the cheese.

METHODS

The colorimetric method of Prill and Hammer (10) seemed to offer the best means of determining the relatively small diacetyl content of cheese. This method was very sensitive and could be adapted easily to routine analysis. To adapt this method to the determination of diacetyl in cheese, alterations which involved the preparation of the sample for analysis, a slight modification of the distillation apparatus and measurement of the concentration of ammono-ferrous dimethylglyoximate by means of a Beckman spectrophotometer were made.

Preparation of the cheese sample. Cheese, unlike butter cultures, cultured milks and butter, does not lend itself readily to steam distillation. Upon heating,

Received for publication January 12, 1949.

¹ This work was supported in part by a grant from the National Cheese Institute.

the cheese becomes a plastic mass with a tendency to retain any volatile components ordinarily removed by steam distillation of a liquid. Even after grinding, all of the diacetyl that is present cannot be removed from the cheese unless the sample is converted into a liquid form.

Consistent results can be obtained if the sample of cheese is treated in the following manner: A given weight of ground cheese (usually 200 g.) plus an equal weight of distilled water are placed in a Waring blender. The blender is run at low speed for 2 min., followed by mixing at high speed for 3 min. Such treatment produces, for all practical purposes, a homogeneous suspension, as the data presented in table 1 indicate. The differences between the theoretical solids

TABLE 1

Solids measurements of cheese-water mixtures as a criterion of homogeneity

Sample no.	Cheese	Solids/100 g. cheese-water mixtures ^a		Difference	Cheese equivalent to the difference
		Measured	Calculated		
	(g.)	(g.)	(g.)	(g.)	(g.)
1	65.05	32.90	32.53	+ 0.37	0.57
2	65.50	32.60	32.75	- 0.15	0.29
3	64.95	32.80	32.47	+ 0.33	0.51
4	65.40	33.00	32.70	+ 0.30	0.46
5	64.60	32.80	32.35	- 0.45	0.68

^a Solids content determined by *Official Methods* (1).

content of the cheese-water mixture, and the measured solids content were converted by calculation to their equivalent weights of cheese. The standard deviation of these differences was calculated and found to be equivalent to ± 0.38 g. of cheese. The maximum difference rarely should exceed three times the standard deviation or approximately ± 1.14 g. of cheese when a 200 g. aliquot of the cheese-water mixture is used to represent 100 g. of cheese.

Subsequent analyses indicated that only rarely would the diacetyl content of cheese exceed 0.1 mg. per 100 g. Therefore, the error in the amount of diacetyl measured by using this procedure would not exceed ± 0.001 mg. This amount of diacetyl is so small that it would not affect materially the final results of the determination.

Modification of distillation apparatus. The distillation apparatus used for the determination of diacetyl in cheese was essentially the same as described by Prill and Hammer (10). However, instead of using glass discs in the reflux-fractionating column, the columns were packed with small pieces of 2 mm. glass tubing, about 5 to 10 mm. in length.

A simple steam generator was made to insure a constant source of uncontaminated steam. This generator was constructed in the following manner: Eleven ft. of nichrome wire with an approximate total resistance of 24 ohms was wound in the form of a coil and placed in the bottom of a 3-l. round-bottomed flask. Each end of the wire was connected to a copper lead, sealed in glass tubing that passed through the rubber stopper. The other end of the copper lead was con-

nected to a rheostat connected to the 110-volt, A.C. outlet to control the flow of steam. A glass funnel with a section of rubber tubing and a pinchcock in the stem was passed through the rubber stopper. This provided a means of introducing distilled water into the flask. A suitable outlet for the steam and a glass steam trap to collect the condensate also were provided. A steam generator of this design furnished an ample supply of steam for all distillations.

Dimethylglyoxime was used as the diacetyl standard as originally proposed by Prill and Hammer (10).

Spectrophotometric measurement of color. By measuring the intensity of the rose-red color complex with the Beckman spectrophotometer only one or two standards were needed for each group of determinations, rather than the range of standards mentioned in the original procedure.

The absorption spectrum was established by measuring the transmittance for a particular concentration of the diacetyl standard at various wave lengths. The results were plotted in the form of a curve relating optical density to wave length. For these determinations a Beckman spectrophotometer (model DU) and 1.000 cm. Corex cells were used. A nominal band width of 1 to 2 $m\mu$. was used throughout the absorption spectrum determinations. The optical density was read directly from the spectrophotometer.

Solutions containing the equivalent of 0.02, 0.05 and 0.10 mg. of diacetyl per ml. were made from the diacetyl standard. The colored ammonio-ferrous dimethylglyoximate derivative was prepared from each of these solutions according to the procedure of Prill and Hammer (10). A blank determination on all reagents was prepared at the same time. The optical density of these solutions was measured at various wave lengths ranging from 500 to 560 $m\mu$. Maximum absorption was obtained at 530 $m\mu$. Within the range of concentrations tested, Beer's law is applicable for this determination.

The accuracy of this modification of the Prill and Hammer method for the determination of diacetyl in Cheddar cheese was determined by means of recovery tests. To samples of each cheese, which had been analyzed previously for diacetyl by the modified method, known quantities of purified diacetyl were added. The total diacetyl content of these cheese-diacetyl mixtures then was determined by the modified method. The results of these recovery tests are shown in table 2. The percentages of recovery ranged from 95 to 102.3 per cent. The method appeared to be of sufficient sensitivity to measure the diacetyl content of cheese.

This modified colorimetric procedure was used for the diacetyl determinations of all lots of cheese. Duplicate samples of each cheese were analyzed. Samples were obtained by grinding either the whole cheese or a wedge of the cheese as described in *Official Methods* (1).

EXPERIMENTAL RESULTS

Twenty-eight lots of Cheddar cheese and eight lots of other varieties of cheese were selected for this investigation. The majority of the lots of Cheddar cheese had been manufactured at the Dairy Department of the University of Wisconsin. The rest of the lots were manufactured at various plants within the state of Wis-

consin. Typical cheese was selected so that it would resemble the average cheese found on the commercial market. Usually little was known concerning the making process of any particular lot of cheese. Fourteen lots of the Cheddar cheese had been manufactured from pasteurized milk, the remainder from raw milk. The cheese ranged in age from 1 to 48 months. Each lot of Cheddar cheese was examined by a judging panel composed of from one to three competent cheese judges. Comments upon the flavor of each lot of cheese were made by each judge. The flavor criticisms used were the standard comments usually employed in evaluating the quality of a cheese on the basis of flavor (8).

TABLE 2
Recovery tests

Test no.	Diacetyl in cheese	Diacetyl added	Diacetyl recovered	Recovery
	(mg.)	(mg.)	(mg.)	(%)
1	0.026	0.009	0.034	97.25
2	0.026	0.018	0.045	102.30
3	0.026	0.027	0.052	98.20
4	0.026	0.045	0.069	97.20
5	0.026	0.090	0.111	95.70
6	0.035	0.017	0.050	96.30
7	0.035	0.034	0.068	98.75
8	0.035	0.051	0.084	97.80
9	0.035	0.068	0.100	97.23
10	0.035	0.085	0.114	95.00

Other varieties of cheese included in this investigation with the diacetyl content of each lot (expressed as mg. per 100 g.) were as follows: Swiss (0.036), Brick (0.017), Limburger (0.021), Camembert (0.008), Blue Mold (0.030), Edam (0.043), Port Salut (0.074) and Cottage (0.029).

To illustrate the variations in the diacetyl content of the different lots of Cheddar cheese, the 28 lots were classified according to the diacetyl content. The results of this grouping are shown in table 3. The majority of all lots contained less than 0.05 mg. per 100 g. of cheese.

TABLE 3
*Diacetyl in cheddar cheese**

Diacetyl content (mg./g. of cheese)	No. of lots	% of lots
0.000-0.049	16	57.2
0.050-0.099	5	17.8
0.100-0.149	2	7.2
0.150 and over	5	17.8

* The diacetyl content of the Cheddar cheese ranged from 0.016 to 0.335 mg./100 g.

Flavor characteristics were used to divide the 28 lots of Cheddar cheese into two groups. One group of "Excellent Flavor" consisted of lots which had no adverse flavor criticisms. The other group contained all lots that had adverse flavor criticisms. These adverse flavor criticisms included anything from a very

slight off-flavor to a definitely objectionable flavor. This latter group was called the group with "All Other Flavors." Each of these two flavor groups then was divided into two sub-groups based on the diacetyl content of the lot. One sub-group contained all lots with less than 0.05 mg. of diacetyl per 100 g. of cheese. The other sub-group consisted of all lots with more than this amount of diacetyl.

The number of lots in each sub-group then was expressed as the per cent of the total lots in that flavor group. Seventy-nine per cent of the lots in the group with "Excellent Flavor" had a diacetyl content of less than 0.05 mg. per 100 mg. In the group with "All Other Flavors" 63 per cent of the lots had more than this amount of diacetyl.

DISCUSSION

The experimental data have shown that diacetyl was present in cheese both when it had an excellent flavor and when it was criticized adversely for flavor. The majority of the lots of cheese with an excellent flavor had a diacetyl content of less than 0.05 mg. per 100 g. of cheese. On the other hand, most of the lots of cheese with adverse flavor criticisms had a larger diacetyl content than this. The production of diacetyl probably was associated with other biological activities that produced substances that detract from the flavor of a cheese. The presence of these substances, and not the diacetyl content, probably was responsible for the adverse criticisms of the cheese flavor. The data show that it was possible to have more than 0.05 mg. of diacetyl per 100 g. of cheese without the production of the "flavor detracting" substances. Probably there is a maximum diacetyl content which, if exceeded, will cause adverse flavor criticisms. Frequently cheese judges identify the flavor as "acidic" when the cheese is normal in hydrogen ion concentration and has no acid defects in body. Crocker (2) described the flavor of diacetyl as "pseudo-sour." It is highly probable that this "pseudo-sour" flavor of diacetyl gives the impression of "acidic" flavor in a cheese that shows no other signs of high acidity.

Diacetyl seems to be an essential part of the flavor complex of Cheddar cheese. In amounts smaller than 0.05 mg. per 100 g. of cheese it is associated with excellent flavor qualities, but amounts in excess of this appear to be associated with adverse criticisms.

SUMMARY

Diacetyl is a prominent element in the flavor of many food products. To determine its presence in cheese and to make quantitative measurements of it, the colorimetric method described by Prill and Hammer (10) was modified. The color of the ammonio-ferrous dimethylglyoximate formed in this procedure was measured spectrophotometrically.

Diacetyl was found in all lots and kinds of cheese examined. The majority of the lots contained less than 0.05 mg. of diacetyl per 100 g. of cheese. The diacetyl content of Cheddar cheese ranged from 0.016 mg. to 0.335 mg. per 100 g. of cheese.

A small quantity of diacetyl probably contributes to the typical flavor of Cheddar cheese. The majority of the lots of Cheddar cheese with an excellent

flavor had a diacetyl content of less than 0.05 mg. per 100 g. Larger amounts of diacetyl than this frequently appear to be associated with flavor defects.

REFERENCES

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and Tentative Methods of Analysis. 6th ed. Washington, D.C. 1945.
- (2) CROCKER, E. C. Flavor. McGraw-Hill Book Co., Inc., New York, N.Y. 1945.
- (3) CSISZÁR, J., AND BAKOS, A. Acetoin-biacetyl in Cheese. II. Formation of Acetoin-biacetyl in Cheeses. *Tejgazdaság*, 1: 94-9. 1941. *C.A.*, 42: 2688. 1948.
- (4) CSISZÁR, J., BAKOS, A., AND TOMKA, G. Acetoin-biacetyl in Cheese. I. Content in Various Cheeses. *Tejgazdaság*, 1: 56-62. 1941. *C.A.*, 42: 2688. 1948.
- (5) CSISZÁR, J., BAKOS, A., AND TOMKA, G. Acetoin-biacetyl in Cheese. III. Correlation of Quality and Acetoin-biacetyl Content of Cheese. *Tejgazdaság*, 2: 74-9. 1942. *C.A.*, 42: 2688. 1948.
- (6) DAVIES, W. L., DAVIS, J. G., DEARDEN, D. V., AND MATTICK, A. T. R. Studies in Cheddar Cheese. V. The Effect of Chemical Substances on the Ripening Process. *J. Dairy Research*, 7, 2: 92. 1936.
- (7) DAVIS, J. G. The Flavor of Cheese. *Flavours*, 4, 3: 23. 1941.
- (8) NELSON, J. A., AND TROUT, G. M. Judging Dairy Products. 2nd ed. Olsen Publishing Co., Milwaukee, Wis. 1948.
- (9) PRILL, E. A., FABRICIUS, N. E., AND HAMMER, B. W. Diacetyl and Other Alpha Dicarbonyl Compounds with Special Reference to the Flavor of Butter. *Iowa Agr. Expt. Sta. Research Bull.* 268. 1939.
- (10) PRILL, E. A., AND HAMMER, B. W. A Colorimetric Method for the Microdetermination of Diacetyl. *Iowa State Coll. J. Sci.*, 12: 3385. 1938.

A STUDY OF THE DIACETYL IN CHEESE

II. THE CHANGES IN DIACETYL CONTENT OF CHEDDAR CHEESE DURING MANUFACTURING AND CURING¹

HAROLD E. CALBERT AND WALTER V. PRICE
Department of Dairy Industry, University of Wisconsin

A previous study has shown that diacetyl was present in all lots of Cheddar cheese analysed (3). In many of the lots the amount of diacetyl present in the cheese was much greater than could be accounted for by the diacetyl content of the original milk and starter culture from which the cheese had been made. Since the diacetyl content of Cheddar cheese has been related to the flavor of the cheese and appears to be an element of cheese flavor, a further study of the diacetyl in Cheddar cheese seems advisable.

The purpose of this investigation was to measure the development of diacetyl during the manufacturing and early stages of curing of both pasteurized and raw-milk Cheddar cheese.

METHODS

Manufacturing procedures. Six lots of Cheddar cheese were made on six different days. Three lots were manufactured from milk pasteurized at 144° F. for 30 minutes. The other three lots were made from raw milk which had a reduction time of 4 hr. on the standard methylene blue test (1). The method of Price (11) was followed for all making operations.

Two lots of identical milk were made into Cheddar cheese, the milk being pasteurized for one lot and raw for the other. These lots were used to study the diacetyl content of cheese during the early stages of curing. The curd was placed in square hoops, pressed, cut into 2-lb. blocks, then pressed again overnight. The blocks of cheese were removed from the press the following morning and placed on shelves in the curing room which was maintained at 60 to 62° F. and at a relative humidity of 80 to 85 per cent. After 2 days, when the surfaces were sufficiently dry, each block of cheese was dipped into paraffin and packed in a wooden box. Each wooden box contained 10 of the 2-lb. blocks of cheese. The boxes were covered to minimize drying of the cheese and were stored at an average temperature of 45° F.

Sampling procedure. In the six lots used to study the development of diacetyl during the making process, the diacetyl content of the starter and of the milk was measured. Samples were removed for diacetyl determinations at setting, cutting, dipping (curd and whey) and milling (curd and whey).

Blocks of the two lots of cheese used to study the diacetyl content during the early stages of curing were ground and duplicate aliquot samples were prepared from each block.

Received for publication January 12, 1949.

¹ This work was supported in part by a grant from the National Cheese Institute.

The diacetyl content of all samples was determined by the modified colorimetric procedure (8).

EXPERIMENTAL RESULTS

Development of diacetyl during the manufacturing procedure. The diacetyl measurements at the various steps during the making of the 6 lots of Cheddar cheese are shown in table 1. The diacetyl contents of the three lots

TABLE 1
Diacetyl measurements during the making of six lots of Cheddar cheese

Material	Diacetyl					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
	(mg./100 g.)					
Milk	0.002	0.002	0.002	0.003	0.002	0.002
Starter	0.037	0.085	0.115	0.040	0.037	0.070
Milk at setting	0.009	0.012	0.015	0.005	0.006	0.007
Curd before cutting	0.010	0.013	0.017	0.005	0.007	0.008
Dipping						
Curd	0.034	0.042	0.021	0.024	0.037	0.031
Whey	0.007	0.015	0.015	0.007	0.008	0.010
Milling						
Curd	0.035	0.047		0.014	0.030	0.058
Whey		0.023	0.022	0.024		0.030

of raw milk at setting were lower than those of the three lots of pasteurized milk. This also was true of the measurements made of the diacetyl in the curd just prior to cutting.

Lots 2 and 4 were used as typical lots to calculate the quantity of diacetyl produced during the making of pasteurized-milk and raw-milk Cheddar cheese. The amount of diacetyl present at each step during the making procedure was calculated as the total milligrams of diacetyl present in either the weight of milk in the vat or the combined weights of curd and whey.

The quantity of diacetyl produced during the making of lot 2, a pasteurized-milk type of Cheddar cheese, was as follows: from the start of the making and up to the time of setting, 19.44 mg.; from setting to cutting, only 2.15 mg.; from cutting to dipping, 12.50 mg.; and from dipping until milling, 11.90 mg. The total increase of diacetyl was 45.99 mg.

A total of 39.16 mg. of diacetyl was produced during the making of lot 4, a raw-milk type of Cheddar cheese. Of this total, 1.42 mg. was produced prior to setting; none from setting to cutting; 9.50 mg. from cutting to dipping; and 28.74 mg. from dipping to milling.

Diacetyl content during the early stages of curing. The diacetyl content of a sample from a block of cheese representing each vat lot was determined at pressing and at 3, 18, 28, 34 and 45 days after pressing.

The gradual decrease in the diacetyl content of each type of Cheddar cheese during the early stages of curing is shown in table 2.

DISCUSSION

It is very likely that the diacetyl which is in cheese originates in a manner comparable to its formation in starter cultures. In starter cultures diacetyl is formed by bacteria acting upon the lactose and citrates. Van Beynum and Pette (13) have described the formation of the diacetyl from the citrates.

Many investigators have shown that diacetyl is a constituent of the desirable flavor and aroma in butter cultures (8). The organisms responsible for the production of this desirable flavor and aroma are *Streptococcus lactis*, *Leuconostoc dextranicum* (*Streptococcus paracitrovorus*) and *Leuconostoc citrovorum* (*Streptococcus citrovorus*) (7). Wilster and Price (15) have pointed out that these same organisms always are present in a good cheese starter as used in this country. It is primarily through their action that the lactose and citrates of the milk might be converted into fermentation products such as diacetyl in the cheese.

TABLE 2
Diacetyl content of Cheddar cheese during early stages of curing

Age	Diacetyl content	
	Pasteurized-milk type	Raw-milk type
(d.)	(mg./100 g.)	(mg./100 g.)
0	0.046	0.035
3	0.042	0.032
18	0.033	0.017
28	0.025	0.021
34	0.025	0.016
45	0.027	0.017

Many workers have shown that aeration and increased acidities favor the production of diacetyl in starter cultures. Prill and Hammer (12) noted that aeration of ripening starter cultures by shaking produced significant increases in the diacetyl, while the lack of aeration caused decreases in the diacetyl. They also noted that lowering the pH of the starter culture tended to increase the diacetyl content. Hedrick and Hammer (9) pointed out that an increased diacetyl content could be obtained in ripening cream by development of higher acidities and use of agitation. Increases up to several hundred per cent in the diacetyl content of starter cultures were noted by Brewer *et al.* (2) when air was bubbled through the starter culture. Cox (4) studied the effect of acidity on the production of diacetyl by betacocci in milk. His investigations were in the pH ranges of 5.5 to 4.4. He found that the rate of growth of the organisms was slower progressively with decreasing pH, but at least as much diacetyl was formed eventually at the low pH values as at high pH values. The results of Cox's investigations showed that there was an alteration in the metabolism of the organisms, the diacetyl producing power per unit cell in-

creasing with decreasing pH. According to van Beynum and Pette (13), the diacetyl was produced from citric acid only when the medium was acid and the conditions aerobic.

The same factors as discussed above also influence the rate of diacetyl production during the making of Cheddar cheese. The experimental data of this study with Cheddar cheese show that while the milk and starter are being stirred, prior to the time of setting, there is an increase in diacetyl. This stirring or agitation provides a means of aeration that would be expected to favor diacetyl production. Little diacetyl is produced between the time of setting and the time of cutting. During this period the material in the vat is quiescent, and the aeration that occurs at other times during the making procedure is lacking. Also, the pH at this time is much higher than it is later in the making procedure. This combination of factors does not encourage diacetyl production. From the time of cutting until the time of dipping, the material in the vat is stirred continuously. This aeration favors the production of diacetyl. From the time of dipping until milling diacetyl production is favored by the rapidly decreasing pH in the curd and whey.

In the six lots of cheese used in this investigation, the rate of diacetyl production during the early steps of the making procedure appears to be slower in the raw-milk than in the pasteurized-milk Cheddar cheese. The nature of the experimental procedure does not establish this as an actual fact. The trend is most interesting and might be investigated further because it suggests the action or influence of: (a) either substances or microorganisms in the raw milk that slow down or retard the production of diacetyl by the "aroma-formers" until later in the making procedure, (b) lack of oxygen or a lower oxidation-reduction potential in the raw milk that prevents or slows down the formation of diacetyl.

The microorganisms responsible for the formation of diacetyl in starter cultures probably are present during the making of cheese. Factors that influence the formation of diacetyl in starter cultures undoubtedly also influence its formation in cheese. Therefore, the diacetyl formed during the making of Cheddar cheese probably comes from the same source and is produced in the same manner as the diacetyl produced in starter cultures.

A gradual decline in the diacetyl content of both the pasteurized-milk and raw-milk Cheddar cheese was noted during the early stages of curing. This parallels observations which have been made by several investigators on the decline in the diacetyl content of butter during storage. According to Langton (10), the gradual disappearance of diacetyl in butter is caused by volatilization. On the other hand, many investigators, including Elliker (6), have indicated that some of the diacetyl in butter is reduced to acetylmethylcarbinol and 2,3-butylene glycol by the action of microorganisms. Virtanen and Kontio (14) added known amounts of diacetyl to samples of milk; the milk then was inoculated with cultures of microorganisms that had been isolated from butter. A decline in the diacetyl content of the milk samples was caused by the action of the microorganisms.

These factors responsible for the decline in the diacetyl content of butter during storage also may explain the decline of diacetyl in cheese. Carbon dioxide and moisture are lost in large amounts by volatilization from the surfaces of the cheese in the early stages of curing. This action would be expected to remove volatile substances like diacetyl from the cheese. The changes in the oxidation-reduction potential of the cheese during curing (5) may cause the diacetyl to undergo chemical changes. Undoubtedly, the microorganisms present in the cheese during this period are responsible in part for the decline in diacetyl. The decline of the diacetyl content in the cheese probably can be attributed to a combination of these physical, chemical and biological influences.

SUMMARY

Diacetyl is produced throughout the manufacturing process of Cheddar cheese except during the interval between setting and cutting. Agitation and increased acidity were factors associated with the increase in diacetyl content.

The diacetyl in Cheddar cheese probably is produced by the action of microorganisms upon the citrates in a manner similar to its production in starter cultures.

A gradual decrease in the amount of diacetyl in both raw-milk and pasteurized-milk Cheddar cheese was noted during the early stages of curing. This decrease probably is caused by volatilization of the diacetyl and by its reduction to acetylmethylcarbinol and 2,3-butylene glycol as a result of chemical reaction or the action of microorganisms.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. *Standard Methods for the Examination of Dairy Products*. 8th ed. 1941.
- (2) BREWER, C. R., MICHAELIAN, M. B., WERKMAN, C. H., AND HAMMER, B. W. The Action of Air Under Pressure in the Oxidation of Acetylmethylcarbinol to Diacetyl in Butter Cultures. *J. Bact.*, 23: 92. 1937.
- (3) CALBERT, H. E., AND PRICE, W. V. A Study of the Diacetyl in Cheese. I. Diacetyl Content and Flavor of Cheddar Cheese. *J. Dairy Sci.*, 32, 6: 515-520. 1949.
- (4) COX, G. A. The Effect of Acidity on the Production of Diacetyl by *Betacocci* in Milk. *J. Dairy Research*, 14: 28-35. 1945.
- (5) DAVIS, J. G. Studies in Cheddar Cheese. I. The Oxidation-Reduction Potentials of Ripening Cheddar Cheese. *J. Dairy Research*, 3, 2: 241-253. 1932.
- (6) ELLIKER, P. R. Effect of Various Bacteria on Diacetyl Content and Flavor of Butter. *J. Dairy Sci.*, 28: 93-102. 1945.
- (7) FABRICIUS, N. E. The Manufacture and Use of Butter Culture. *Dairy Ind.*, 5: 159-60. 1940.
- (8) HAMMER, B. W., AND BABEL, F. J. Bacteriology of Butter Cultures. A Review. *J. Dairy Sci.*, 26, 2: 83-168. 1943.
- (9) HEDRICK, T. I., AND HAMMER, B. W. Diacetyl and Acetylmethylcarbinol Production in the Manufacture of Unsalted Butter. *Iowa Agr. Expt. Sta. Research Bull.* 301. 1942.
- (10) LANGTON, H. M. Recent Work on Flavor in Butter and Other Fats. *Food*, 7: 315-16. 1938.

- (11) PRICE, W. V. Cheddar Cheese from Pasteurized Milk. Wis. Agr. Expt. Sta. Bull. 464. 1944.
- (12) PRILL, E. A., AND HAMMER, B. W. Production of Diacetyl from Citric Acid in Butter Cultures. J. Dairy Sci., 22: 67-77. 1939.
- (13) VAN BEYNUM, J., AND PETTE, J. W. The Decomposition of Citric Acid by *Betaoococcus cremoris*. J. Dairy Research, 10: 250-66. 1939.
- (14) VIETANEN, A. I., AND KONTIO, P. Disappearance of Acetylmethylcarbinol and Biacetyl Caused by Microorganisms. Ylipainos Karjautuotteesta, 8. 1939. (Dairy Sci. Abstracts, 2, 1: 52. 1940.)
- (15) WILSTER, G. H., AND PRICE, F. E. Preparation of Starter for Cheese, Buttermilk and Butter. Oregon Agr. Expt. Sta. Bull. 379. 1940.

COBALT TOLERANCE IN YOUNG DAIRY CATTLE¹

H. A. KEENER, G. P. PERCIVAL AND K. S. MORROW
New Hampshire Agricultural Experiment Station, Durham

AND

G. H. ELLIS

U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y.

Cobalt deficiency has been reported to be rather widespread in the United States and Canada, as well as in various other parts of the world. In spite of the fact that numerous publications which deal with various phases of this deficiency are available, little information on the tolerance of ruminants to the feeding of this element has been found. Josland (5) reported from New Zealand in 1937 that when four ewes were drenched daily for 7 months with 1 mg. of cobalt (from the sulfate) per 200 g. body weight, one developed a polycythemia within 3 months, two became mildly anemic and one was not affected. No toxic effects were observed. In 1945, Geyer *et al.* (4) reported that the tolerance of the bovine to cobalt is high and that feeding as much as 50 mg. per day did not produce polycythemia. Ely *et al.* (2) reported in 1946 that there are relatively wide variations in the tolerance of individual animals to high doses of cobalt salts. A detrimental effect on the appetite was reported for animals which received excessive cobalt orally, while excessive intravenous injections produced rapid respiration, incoordination, lacrimation, salivation, defecation and urine leakage within 1 to 5 minutes after injection. Very recently Ely *et al.* (3) reported that equivalent amounts of cobalt fed as the sulfate, chloride or carbonate were equally toxic to the dairy calf if fed in amounts in excess of 40 mg. daily per 100 lb. of body weight.

After Keener *et al.* (6) reported in 1944 that cobalt deficiency existed in New Hampshire, further results of these workers as well as the experiences of farmers, veterinarians, and feed dealers showed that the deficiency was widespread in that state. Since it was obvious that the feeding of supplemental cobalt would become a common practice, first-hand information on the tolerance of the bovine to cobalt seemed necessary. The experiment reported here, a preliminary report (7) of which was given in 1947, was undertaken for that purpose.

EXPERIMENTAL PROCEDURE

This study was started in September, 1944, and the last animals were removed from the experiment on January 1, 1947. All animals were either purebred or high grade Holsteins. Those calves which were placed on the experiment at an early age were changed from whole milk to reconstituted skim milk at 1 to 2 weeks of age and received this until 6 to 10 weeks of age. They were fed a concentrate mixture consisting of 3 parts of ground corn, 3 parts of ground oats, 3 parts of

Received for publication January 20, 1949.

¹ Scientific contribution no. 124 of the New Hampshire Agricultural Experiment Station.

wheat bran, 1 part of soybean oil meal and 1 per cent salt throughout the time they were on experiment. The roughage fed was a grass hay of average quality. Water was furnished twice per day in individual pails to prevent the control animals from receiving cobalt. Cobaltous sulfate (C. P.) was used as the source of cobalt. It was fed to the first group of animals once per day in the drinking water. All later animals were given cobalt twice a day in the same manner except

TABLE 1
Summary of level and duration of cobalt feeding and the weight and health of calves

Animal	Sex	Age at begin- ning	Time on experi- ment	Cobalt level	Body weight		General condition
					Begin- ning	Gain	
		(wk.)	(wk.)	(mg./d./100 lb. body wt.)	(lb.)	(lb./d.)	
<i>GROUP I</i>							
43	M	14	28	100*	228	2.14	Normal
			13	400*	648	1.71	Indications of hyperchromemia
			16	1000*	804	0.87	Rough coat, lacked muscular coordination, improved some at end of period. Hyperchromemia
44	F	12	28	25*	181	1.65	Normal
			13	200*	505	1.90	Normal
			16	500*	678	1.03	Normal
			14	none	794	0.84	Normal
			4	2000*	876	-0.96	Off feed at intervals, hyperchromemia
			21	none	849	1.30	Returned to normal after few wk.
45	F	4	31	none	102	1.71	Normal
<i>GROUP II</i>							
77	M	2	10	none	90	1.26	Normal
			11	none	178	1.31	Normal
			20	none	279	1.41	Normal
			13	none	476	1.47	Normal
78	M	1	7	none	610	1.77	Normal
			9	none	86	1.30	Normal
			11	50	168	1.04	Indications of hyperchromemia
			20	10	248	1.54	Normal
79	M	1	13	50	464	1.64	Normal
			7	100	613	1.41	Indications of hyperchromemia
			7	none	86	1.02	Normal
			11	90	136	0.97	Indications of hyperchromemia
			20	30	211	1.47	Normal
			13	30	417	1.88	Normal
			7	75	588	0.77	Indications of hyperchromemia

* Total cobalt fed daily.

in those instances where the amount was so large that the animals would not drink the water containing it. In this case it was given as a drench twice a day. Calves were kept in individual pens. Weights, heights at withers and chest circumferences were determined weekly. Hemoglobin determinations were made periodically on each animal throughout the experiment. Packed red cell volume determinations were made on all but the first group. The animals in groups II and

IV were slaughtered at the end of the experiment so tissues could be taken for cobalt analysis.

RESULTS

A summary of the rates of cobalt feeding and the results obtained with each animal is given in table 1. The animals in group I were used in preliminary studies and cobalt was not fed according to body weight. However, cobalt was

TABLE 1—(Continued)

Animal	Sex	Age at begin- ning	Time on experi- ment	Cobalt level	Body weight		General condition
					Begin- ning	Gain	
		(wk.)	(wk.)	(mg./d./100 lb. body wt.)	(lb.)	(lb./d.)	
GROUP III							
80	F	40 (est.)	14	none	473	1.40	Normal
			23	30	610	1.42	Normal
			13	30	838	1.43	Normal
			7	75	968	0.16	Normal
81	F	42 (est.)	13	none	498	1.23	Normal
			23	30	610	1.27	Normal
			13	50	815	1.32	Indications of hyperchromemia
			7	100	935	0.12	Slight hyperchromemia
			GROUP IV				
82	M	2	4	none	92	1.50	Normal
			11	none	134	1.05	Normal
			20	none	215	1.60	Normal
			13	none	439	1.86	Normal
			7	none	608	0.75	Normal
83	M	1	4	none	81	1.11	Normal
			11	50	112	0.75	Indications of hyperchromemia
			20	10	170	1.48	Normal
			13	50	378	1.46	Normal
			6	100	511	-0.21	Listless, poor appetite, unsteady gait, hyperchromemia
84	M	2	1	none	502	-2.43	Some improvement in condition
			4	none	127	1.07	Normal
			11	90	157	0.82	Slight hyperchromemia
			20	30	220	1.51	Returned toward normal
			13	30	431	1.26	Normal
			7	75	546	1.04	Listless, slight hyperchromemia

fed to all other animals in proportion to body weight. With the remaining calves, there were three groups based on age and period on the experiment. In both groups II and IV, a negative control was maintained. Group III consisted of two animals placed on the experiment at an older age.

Figure 1 shows the effect of the level of cobalt intake on the hemoglobin content of the blood. Since the values obtained for packed red cell volume parallel rather closely those obtained for hemoglobin, the packed red cell volume values are not given.

Because of differences in the response of various animals, it was not possible to determine accurately the maximum rate of cobalt feeding which would not produce deleterious effect. However, cobalt in the form of cobaltous sulfate did not produce any apparent harmful effects until fed at a rate approaching 50 mg.

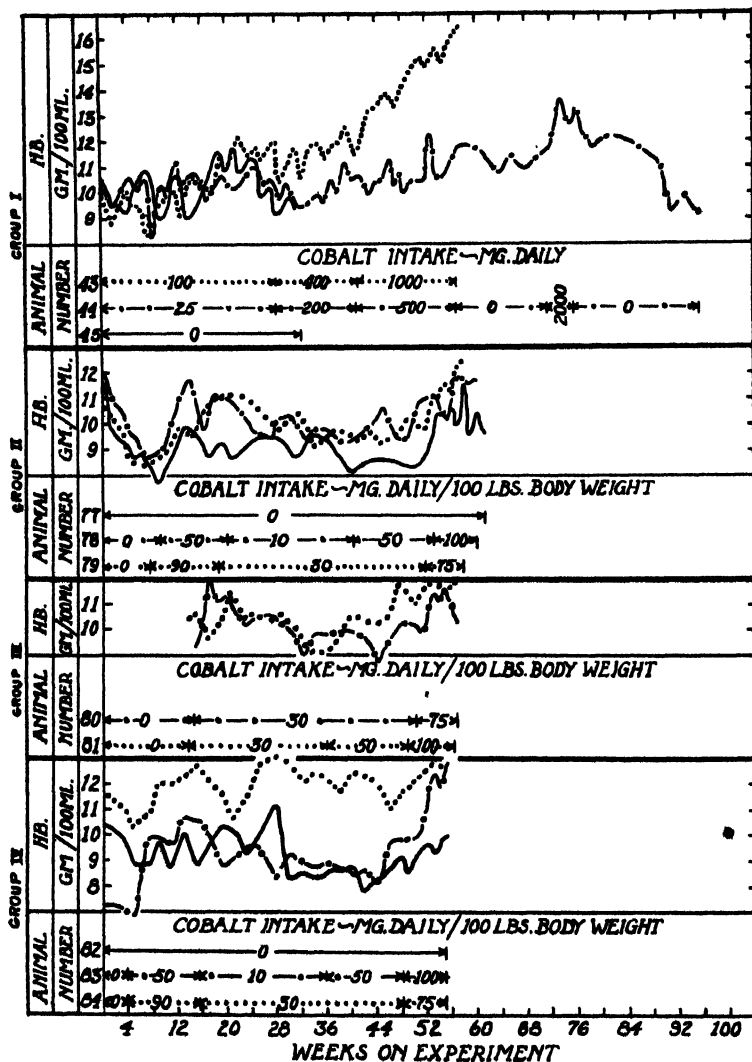


FIG. 1. Curves showing hemoglobin levels and rates of cobalt feeding for the various calves.

daily per 100 lb. body weight for a period of many weeks. With number 84, the hemoglobin and packed red cell volume levels appeared to be a little higher than normal, if compared to values obtained from the other calves. However, if one

considers hemoglobin values of 10.9 ± 0.86 g. per 100 ml. for cows and 12.8 ± 0.8 for bulls as given by McCay (8) to be normal for these animals, neither number 84 nor any of the other animals could be considered to show hyperchromemia except at the highest levels of cobalt intake. In determining the earliest symptoms of excess cobalt consumption, hemoglobin and packed red cell volume changes for each period were given as much consideration as the actual hemoglobin and packed red cell volume levels.

When cobalt levels of 50 mg. daily per 100 lb. of body weight were fed for periods of many weeks, there usually was an increase in hemoglobin and packed red cell volume. This increase was almost imperceptible in some cases and very marked in others. The relationship between the level of cobalt intake and the increase in body weight and height at withers was in line with the conclusions drawn from the blood studies. No depressing effect on growth was noted on any animal which received less than 50 mg. of cobalt per 100 lb. of body weight per

TABLE 2
Cobalt content of kidney and liver tissue

ANIMAL NO.	SAMPLE ^a	Cobalt content of:	
		Kidney	Liver
		(γ /g. dry wt.) ^b	(γ /g. dry wt.) ^b
77 (control)	a	0.26	0.49
	b	0.31	0.44
78	a	2.4	2.1
	b	2.5	3.3
79	a	1.9	7.1
	b	2.4	7.0
82 (control)	a	0.41	0.85
	b	0.33	0.61
83	a	4.9	
	b	5.4	4.4
84	a	4.2	15.4
	b	2.2	4.3

^a Taken from different parts of organ.

^b Av. of duplicates in most cases.

day. However, there was a depressing action in some cases when cobalt was fed at higher levels and particularly when more marked effects were noted in the blood and externally. With the most severe external symptoms, a loss of weight was observed.

Definite external effects of excessive cobalt consumption were observed only on a few animals which had been fed at rates approaching at least 100 mg. of cobalt per 100 lb. of body weight per day for many weeks. One animal showed some effect in 4 weeks when fed a little over twice this amount. These external effects were rough hair coat, listlessness, depressed appetite, decreased water consumption and lack of muscular coordination. In fact, they appeared to be in general almost the same as for cobalt deficiency. However, the blood picture was quite the opposite.

The effect of feeding relatively large amounts of cobalt over considerable periods of time on the storage of cobalt in the tissues of the liver and kidneys is shown in table 2. Although the cobalt content of these tissues from the animals fed cobalt was several times as high as those from the controls, it is still relatively low when one considers the amounts of cobalt fed and the fact that the results were expressed on the basis of dry weight. Since, according to the data of Comar and Davis (1) orally administered cobalt is stored to a much greater extent in the liver and kidney than in most other organs of the body, it would appear that there is little possibility that excessive concentrations of cobalt will be stored in the various organs of the body when the animal is fed this element at the usual rates.

The results obtained in this experiment indicate considerable variation in the response of different animals to high rates of cobalt feeding. The rates of feeding generally followed allow a wide margin of safety. The practice usually followed by feed manufacturers in this area of supplementing with approximately 2 g. of cobalt sulphate or equivalent per ton of mixed feed generally furnishes less than 1 per cent of the tolerance level determined in this experiment.

SUMMARY

1. An experiment was carried out with growing Holstein dairy cattle to determine the amount of cobalt they can consume continuously with safety over a considerable period of time.
2. There was considerable individual variation in the tolerance level.
3. The oral consumption of a small excess of cobalt sulfate produced an increase in hemoglobin and packed red cell volume.
4. The oral consumption of a greater excess of cobalt sulfate resulted in loss of appetite, decreased water consumption, rough hair coat and a lack of muscular coordination, as well as an increase in hemoglobin and packed red cell volume.
5. High levels of cobalt as fed during this experiment increased the cobalt content of kidney and liver tissues to several times that of the controls, but in light of the levels fed and the duration of the feeding, these accumulations are considered to be low.
6. Growing dairy animals as maintained on this experiment appeared to be able to consume daily up to approximately 50 mg. of cobalt per 100 lb. of body weight from cobaltous sulfate for many weeks without definite harmful effects.
7. The levels of cobalt generally added to concentrate rations by feed manufacturers and those generally recommended for inclusion in mineral mixtures appear to afford a wide margin of safety.

REFERENCES

- (1) COMAR, C. L., AND DAVIS, G. K. Cobalt Metabolism Studies. IV. Tissue Distribution of Radioactive Cobalt Administered to Rabbits, Swine and Young Calves. *J. Biol. Chem.*, 170: 379-389. 1947.
- (2) ELY, R. E., DUNN, K. M., DUNCAN, C. W., AND HUFFMAN, C. F. Some Factors Affecting the Toxicity of Cobalt in the Bovine. *J. Animal Sci.*, 5: 398. 1946.

- (3) ELY, R. E., DUNN, K. M., AND HUFFMAN, C. F. Cobalt Toxicity in Calves Resulting from High Oral Administration. *J. Animal Sci.*, 7: 239-246. 1948.
- (4) GEYER, R. P., RUPEL, I. W., AND HART, E. B. Cobalt Deficiency in Cattle in the North Eastern Region of Wisconsin. *J. Dairy Sci.*, 28: 291-296. 1945.
- (5) JOSLAND, S. W. The Effect of Feeding Excess of Cobalt to Healthy Sheep. *New Zealand J. Sci. Technol.*, 19: 31-37. 1937.
- (6) KEENER, H. A., PERCIVAL, G. P., AND MORROW, K. S. Cobalt Treatment of a Nutritional Disease in New Hampshire Dairy Cattle. *New Hamp. Agr. Expt. Sta. Circ.*, 68. 1944.
- (7) KEENER, H. A., PERCIVAL, G. P., MORROW, K. S., AND ELLIS, G. H. Cobalt Tolerance in Young Dairy Cattle. (Abs.) *J. Dairy Sci.*, 30: 557. 1947.
- (8) McCAY, C. M. The Hemoglobin and Total Phosphorus in the Blood of Cows and Bulls. *J. Dairy Sci.*, 14: 373-378. 1931.

FINAL CREAM QUALITY RESULTING FROM KEEPING DELIVERIES SEGREGATED VERSUS MIXING AS PRACTICED IN BUYING STATIONS^{1, 2}

T. J. CLAYDON AND W. H. MARTIN

Kansas Agricultural Experiment Station, Manhattan

Segregation of farm-separated cream according to grades in the buying station generally is considered to be conducive to higher average quality at the creamery than when different grades of cream are mixed at the time of purchase. This tenet is supported by the dairy laws of various states which specify that lots of first and second grade cream are not to be mixed in the buying stations. Manhart (3), in discussing the requirements of the 4-day plan of cream grading, stated that the mixing of undergrade cream with good cream in the buying station was detrimental to the program.

In some areas, however, relatively little grading or segregation is practiced in the station and cream is mixed indiscriminately as a matter of convenience in handling and shipping. Commonplace as is the recommendation to segregate cream, no published data have been found on the merit of the practice under field conditions. If segregation on a grade basis is an important factor in cream quality, then some system of segregation of individual deliveries of cream should enhance quality further. The study herein reported was initiated to appraise the value of such individual segregation as one of the steps in marketing good quality cream and also to estimate the effect of indiscriminate mixing as practiced in many buying stations.

METHODS

The study was carried on over a period of 11 months from April, 1947, to February, 1948, so that any seasonal influences would be included. All cream used in the study was obtained from deliveries by producers to cream stations under normal operating conditions and generally represented the quality range of cream so marketed in Kansas. Due to the practical difficulties involved, the segregation trials were conducted on small lots of cream under controlled laboratory conditions simulating those encountered in practice.

Sampling procedure. Cream stations were visited on days when receipts were expected to be relatively large, so that the samples obtained would represent a larger proportion of the cream marketed. Three samples were taken directly from the well-stirred cream of each delivery and placed in clean, dry, 6-oz. sample jars. The ladle used for sampling was rinsed in warm water and then in a hypochlorite solution (200 to 300 p.p.m.) after sampling each delivery to minimize contamination from one lot of cream to the next. One sample from

Received for publication January 20, 1949.

¹ Contribution no. 183, Department of Dairy Husbandry.

² This study was supported by a grant from Swift and Company, Chicago, Illinois.

each delivery was placed immediately in ice water. The other two samples were held at station temperature until all samples for the day were obtained.

After sampling, the cream delivered by producers was dumped into 10-gallon cans in the usual manner followed in buying stations where segregation on a grade basis is not practiced. As each 10-gallon can was filled, a record was made of the individual deliveries contained. Samples then were taken from the full cans in the same manner as from the separate lots of cream delivered by producers, one being placed in ice water. Thus the samples from the 10-gallon cans contained mixed cream representing deliveries from several patrons. At the end of the buying day all cream samples were returned to the laboratory. The iced set of samples was held iced until analyzed, usually a 16- to 24-hour period. The other two sets of samples were placed at different holding temperatures.

Holding temperatures. In view of the fact that much cream receives little cooling after it has been purchased in the station, consideration was given to the effect of holding conditions on the comparative quality of segregated and mixed cream. A temperature of 80° F. was considered to be an approximate average at which cream often is held during hot weather. As representative of conditions that might be attained where effective cooling is provided, 50° F. was selected. These two temperatures were used in the investigation for holding the cream. It should be noted that holding at the different controlled temperatures started at the end of the buying day to simulate different handling practices that might be applied at that time. The procedure generally conforms to practical conditions since in those stations where cooling facilities are available they frequently are not employed until the end of the buying day. Since the length of time between the purchase of cream at the station and churning at the creamery sometimes extends to 2 days, this period was used for holding the cream samples at the above temperatures.

Examination of samples. To indicate the quality of the cream when received at stations, examinations were made on the iced samples representing both individual deliveries and 10-gallon cans of cream as mixed in stations. As a further indication of average quality, tests were made on a composite sample prepared from the individual samples, representing all cream obtained at a station.

To compare the effect of segregating versus mixing cream, at the end of the holding period in each trial, a composite sample was made of all 10-gallon can samples obtained at the station. This was compared with a proportional composite taken from all individual samples. As a further measure of the effect of segregation, in all but the first three trials, composite samples were prepared from the individual samples representing each 10-gallon can of cream. These composites then were compared with the corresponding 10-gallon can samples.

Quality determinations. Quality was determined on the basis of organoleptic tests supported by titratable acidity, formol titration and mold content. Cream samples were scored for flavor according to the method common in the butter industry where the numerical score is based on the estimated commercial

grade of butter that should be obtained. Such flavor scores were used in preference to grades so that smaller differences in quality could be designated. Samples were scored by two experienced judges working independently. Acidity determinations were made by titrating 9 g. of cream plus 9 ml. of distilled water with 0.1 N NaOH, using phenolphthalein as the indicator. Formol titrations were made by the method used by Martin *et al.* (4) except that 2 ml. of formalin were used instead of 10 ml. The mold content of cream was determined by the Parsons modification (5) of the Wildman methylene blue-borax procedure (6). The mold pads were scored according to a modification of the American Butter Institute tentative mold standard for cream, with the following numerical values being assigned to each grade.

American Butter Institute mold standard: Good : Fair : Poor : Very Poor :
Corresponding scores used in this study: 1 2 3 : 4 5 6 : 7 8 9 : 10 11 12 :

Mold tests were made on the segregated and mixed cream only after holding.

RESULTS

During the 11-month period of the study 12 different trials were conducted involving cream deliveries from 182 producers delivered at 7 different cream buying stations. The stations were located in five towns in four Kansas counties. The number of deliveries sampled per station contact averaged slightly more than 15 and ranged from 7 to 21.

The quality of the cream obtained at the station before holding as represented by composite samples of the day's purchase in each trial is shown in table 1. The range in flavor scores of individual deliveries of cream is included. Also shown is the comparative quality of segregated and mixed cream as represented by composite samples prepared after holding the cream by each method for 2 days at 50° F. After holding there was little difference between the composite quality of the segregated cream and the mixed cream as indicated by flavor score, titratable acidity, formol titration and mold content. The average change in quality was a reduction of 0.7 in flavor score for both the segregated and mixed cream, with increases in acidity of 0.12 and 0.14 per cent, respectively. In some instances part of these changes probably were initiated during the period in the stations. Although there were inconsistencies in the formol titrations, the average values for both the segregated and mixed cream were near that of the cream before holding. The average mold content was about equal for both holding methods.

After being held 2 days at 80° F., the average composite quality of the segregated cream was little different from the average composite quality of the mixed cream (table 2). However, several trials showed an advantage for the segregated cream. As would be expected, deterioration was more extensive at 80° F. than at 50° F. The average quality change involved a reduction in flavor score of 1.6 for the segregated cream and 1.8 for the mixed cream, with increases in acidity of 0.36 and 0.42, respectively. Although the formol titrations again showed variations, the average increases were almost equal, being 0.56 and 0.62

TABLE 1
Effect on composite quality of holding cream as mixed in cream stations versus segregating and holding separately cream from each delivery

Trial no.	Mo.	No. of deliveries	Range of flavor score	Composite quality before holding				Composite quality after holding 2 d. at 50° F.					
				Flavor score	Titra- table acidity	Formol titra- tion	Segre- gated	Flavor Score	Titratable acidity	Formol titration	Mold score	Segre- gated	Mixed
					(%)	(ml.)			(%)	(ml.)			
1	April	18	88.5-92.5	91			90	90	0.73		3	3	3
2	April	9	89.5-92	91	0.59		90.5	90.5	0.68		2	2	2
3	May	18	90-93	91	0.59		91	91	0.67		6	6	6
4	May	17	89-92	90.5	0.59	2.5	90.5	90.5	0.69	2.4	4	4	4
5	June	21	89-92	91	0.60	2.0	89	89	0.66	3.0	4	4	4
6	June	7	88-91	90	0.68	3.1	90	90	0.75	2.4	4	4	4
7	July	15	89-91.5	90	0.89	2.9	89	89	1.08	2.7	4	4	4
8	Aug.	9	89-93	91.5	0.53	2.4	90	90.5	0.77	3.3	5	5	5
9	Sept.	20	88-91	90	0.83	3.0	90	89.5	0.92	2.9	6	6	6
10	Sept.	19	89-93	91.5	0.64	2.9	90.5	90	0.83	2.8	5	5	5
11	Dec.	14	89.5-92	91.5	0.41	2.4	91	91	0.55	1.8	6	6	6
12	Feb.	15	90.5-93	91.5	0.45	2.0	91	91	0.60	2.5	1	1	1
									0.54	2.1			
Summary		182	88-93	90.9	0.62	2.58	90.2	90.2	0.74	2.58	4.0	4.0	4.2

TABLE 2
Effect on composite quality of holding cream as mixed in cream stations versus segregating and holding separately cream from each delivery

Trial no.	Mo.	No. of deliveries	Range of flavor score	Composite quality						Composite quality after holding 2 d. at 80° F.					
				before holding			Flavor score			Titratable acidity			Formol titration		
				Flavor score	Titra- table acidity (%)	Formol titra- tion (ml.)	Segre- gated score	Mixed score	Segre- gated (%)	Mixed (%)	Segre- gated (ml.)	Mixed (ml.)	Segre- gated (%)	Mixed (%)	Mold score
1	April	18	88.5-92.5	91	(%)	(ml.)	89.5	89	(%)	(%)	(ml.)	(ml.)			
2	April	9	89.5-92	91	0.59		90	90	0.79	0.82			5	5	
3	May	18	90-93	91	0.59-		90	89	0.83	0.82			4	4	
4	May	17	89-92	90.5	0.59	2.5	89.5	89	0.88	0.84	2.8	3.2	5	5	
5	June	21	89-92	91	0.60	2.0	88.5	88.5	0.84	0.88	2.6	2.6	5	5	
6	June	7	88-91	90	0.68	3.1	89	89	0.88	0.88	3.5	3.5	6	4	
7	July	15	89-91.5	90	0.89	2.9	89	89	0.96	1.01	2.9	3.2	4	4	
8	Aug.	9	89-93	91.5	0.53	2.4	89	89	1.44	1.57	3.7	3.7	7	5	
9	Sept.	20	88-91	90	0.53	3.0	88	89.5	1.16	1.24	2.7	2.9	6	7	
10	Sept.	19	89-93	91.5	0.64	2.9	89	88	1.36	1.65	4.2	4.5	8	6	
11	Dec.	14	89.5-92	91.5	0.41	2.4	90	89	1.25	1.34	3.5	3.4	6	5	
12	Feb.	15	90.5-93	91.5	0.45	2.0	89.5	89	0.71	0.72	2.8	2.7			
									0.75	0.70	2.7	2.3	5	7	
Summary		182	88-93	90.9	0.62	2.58	89.3	89.1	0.98	1.04	3.14	3.20	5.6	5.3	

for the segregated and mixed cream, respectively. There was no practical difference in the average mold content.

Trials 4 to 9 inclusive (tables 1 and 2) generally reflect the quality situation existing during the warmer months. Although there were exceptions, as would be expected, the average quality of the cream at the time of purchase was lower during the summer months than during the remainder of the year.

During the study it frequently was evident that the higher quality cream deteriorated a greater proportionate amount than did the lower quality cream. Although this characteristic sometimes is recognized, it often receives little consideration in actual commercial operations. To determine the influence of quality on the extent of deterioration in the segregated and mixed cream the data from trials showing comparisons on a 10-gallon can basis were grouped according to flavor scores of the 10-gallon lots of cream. Such cream scoring 91 and over when purchased comprised the better group and cream scoring under 91 represented the poorer group. These data are summarized in table 3 for both of the holding temperatures and with the average results on all cream included for comparison. The average quality of the better cream is indicated by a flavor score of 91.4, an acidity of 0.52 and a formol titration of 2.41. The poorer quality cream had an average flavor score of 90.1, with acidity and formol titrations of 0.79 and 3.08, respectively.

With the better cream very little difference in quality resulted from segregation or mixing. With the poorer cream the differences were somewhat greater, particularly as shown by acidities and formol titrations of the samples held at 80° F. However, in the quality range involved, the differences appear to be of little practical importance. At 80° F. the better cream showed more deterioration in quality than the poorer cream. The average flavor score of the better cream dropped 2.1 points in both the segregated and mixed cream, compared to corresponding changes of 1.4 and 1.6 in the poorer cream. Although the actual increase in acidity was less, the proportionate increase was greater. At 50° F. the drop in quality was slightly greater for the better cream, but the difference was too small to be of practical significance. Although the better cream deteriorated more at 80° F. than the poorer cream, its average quality after holding 2 days generally was the same as that of the poorer cream held at 50° F.

DISCUSSION

The cream used in the study was obtained under practical conditions and generally represented the quality marketed through cream stations in Kansas. With such cream the principal cause of low quality is deterioration. Although the investigation was conducted on small lots of cream rather than on commercial quantities, the results on segregation and mixing of cream show the comparable effects of these treatments and indicate what might be expected under commercial conditions.

During the study formol titrations on some samples failed to show a consistent relationship to other quality measures. This characteristic has been noted

TABLE 3
Influence of cream quality on extent of deterioration during 2 days holding

Class of cream	No. of deliveries	Range of flavor score	Av. quality before holding		Hold- ing temp.	Average quality after holding								
			Flavor score	Titra- table acidity		Flavor score		Titratable acidity		Formol titration		Mold score		
						Segre- gated	Mixed	Segre- gated	Mixed	Segre- gated	Mixed		Segre- gated	Mixed
All creams	182	88-93	90.9	0.62	2.58	50	90.2	90.2	0.74	0.76	2.58	2.64	4.0	4.2
							89.3	89.1	0.98	1.04	3.14	3.20	5.6	5.3
Better cream	77	89-93	91.4	0.52	2.41	50	90.5	90.4	0.64	0.66	2.53	2.60	3.6	4.5
							89.3	89.3	0.87	0.92	3.00	3.08	5.4	4.9
Poorer cream	58	88-90.5	90.1	0.79	3.08	50	89.5	89.2	0.87	0.93	2.99	3.12	4.8	4.6
							88.7	88.5	1.19	1.33	3.34	3.74	5.4	5.6

* From summaries of tables 1 and 2.

by other workers (2, 4). Conversion of results to a fat-free basis did not give greater uniformity. Inconsistencies were more frequent on composite samples. Nevertheless the over-all average changes in formal titrations generally were in agreement with other quality changes and thereby supported the other measures of quality.

Mold tests also showed some inconsistencies between samples. However, this is not unusual when small lots are involved (1). The over-all average of results was in general accordance with the cream quality. With the recognized influence of such factors as proportionate surface area and size of delivery, it is difficult to predict the influence on mold content of the segregation of individual deliveries under commercial conditions.

The effect on final composite quality of segregating cream from different deliveries to cream stations was of doubtful importance. Under commercial conditions it would appear that any advantages in quality that might be gained would not be sufficient to justify the practical difficulties involved. Where flavor defects, due to plants or foreign materials, are a relatively minor problem, and where cream of reject quality is not involved, it also is doubtful that the manner of mixing cream in the cream station has any important practical effect on the average quality. However, segregation on a grade basis may provide more opportunity for selection at the creamery if temperature control is maintained.

The fact that the better cream showed a greater amount of deterioration than the poorer cream when held at a relatively high temperature is in accordance with general observations under practical conditions as well as with available information on the growth rates of bacteria. The bacterial development in the good cream at the time of purchase probably is in the logarithmic growth phase where changes are potentially rapid, while in the poorer cream activity has reached a peak and further population changes generally are slower. Although the better cream may remain higher in quality than the poorer cream for some time, it undergoes greater deterioration unless holding temperatures are relatively low. Obviously high quality cream requires better care after purchase than low quality cream if further changes are to be kept at a minimum. In commercial practice this situation would seem to merit greater attention. With any improvement in the quality of cream marketed by the producer also must come improvement in subsequent handling if the gains so made are not to be nullified.

Although the study was concerned mainly with the comparative effects of segregation and mixing, the results re-emphasize the importance of temperature in quality changes. It is evident that such practices as segregation or mixing of cream are of minor significance compared with effective temperature control.

CONCLUSIONS

There was little practical difference between the average quality of cream held segregated and cream held as mixed in buying stations when holding conditions involved temperatures of 50 or 80° F. for 2 days following purchase. Most differences were slightly in favor of the segregated cream. Segregation generally had a greater effect on the poorer quality cream. It is considered that

any contribution to quality that might be gained through segregation of deliveries under commercial conditions would be insufficient to justify the practical difficulties involved.

The better quality cream deteriorated proportionately more after purchase than the poorer quality cream when the holding temperature was 80° F. This emphasizes that any improvement in the quality of cream marketed by producers must be accompanied by a corresponding improvement in subsequent holding conditions.

REFERENCES

- (1) CLAYDON, T. J. Some Factors Affecting the Mold Content of Cream and the Mycelia Count of Butter. Ark. Agr. Expt. Sta. Bull. 432. 1943.
- (2) JEZESKI, J. J., AND MACY, H. Cryophilic Organisms in Water and Butter. J. Dairy Sci., 29: 439-452. 1946.
- (3) MANHART, V. C. Cream Grading on the Four Day Delivery Plan in Indiana. Purdue Univ. Agr. Expt. Sta. Circ. 193. 1932.
- (4) MARTIN, W. H., FAY, A. C., AND CAULFIELD, W. J. The Effect of Temperature and Time of Storage of Cream on the Rate and Type of Deterioration. J. Dairy Sci., 20: 667-678. 1937.
- (5) PARSONS, C. H. A Visual Mold Test for Cream and Patron Reaction to It. Natl. Butter Cheese J., 32, 3: 12. 1941.
- (6) WILDMAN, J. D. Development of Methods for the Estimation of Mold in Cream or Butter. J. Assoc. Offic. Agr. Chemists, 20: 93-100. 1937.

BACTERIOLOGICAL STUDIES OF BOVINE SEMEN. I. NUMBERS OF BACTERIA AND THE RELATION TO FERTILITY¹

J. O. ALMQUIST, P. W. PRINCE, AND J. J. REID

Departments of Dairy Husbandry and Bacteriology, Pennsylvania State College, State College

During the past decade the problems associated with the presence of bacteria in bull semen used for artificial breeding have received increased attention. Special emphasis has been placed on the use of various antibacterial agents, including penicillin, streptomycin and various sulfonamides, for controlling the bacteria which always are found in the semen of bulls (2, 3, 5, 8, 9, 11, 13). The effect upon fertility of the addition of either sulfanilamide or penicillin to diluted bull semen used for routine artificial breeding also has been investigated. Salisbury and Knodt (15) reported that sulfanilamide in the diluter gave a significant increase in fertility. Almquist (1) found that penicillin markedly improved the fertility of semen from certain relatively infertile bulls although an earlier report by Almquist *et al.* (4) showed that it did not increase the fertility of semen from bulls of relatively high breeding efficiency. However, there is need for more fundamental information on the numbers of bacteria commonly found in bull semen, particularly the relationship of bacteria to the fertility of bull semen used for artificial breeding. Such information would be of particular value in determining the possible role of bacteria in fertility and in interpreting the results of fertility tests involving antibacterial substances.

Gunsalus *et al.* (10) found that the logarithmic average of plate counts for 15 ejaculates from 12 bulls used for artificial breeding was 22,000 bacteria per ml. Twenty-eight ejaculates from seven bulls used for natural service gave a somewhat higher average logarithmic plate count of 225,000 per ml. Plate counts for the 43 ejaculates collected from 19 bulls by means of the artificial vagina ranged from 1,000 to 22 million bacteria per ml. Other workers (3, 5, 7, 14) have reported bacterial counts of semen collected with the artificial vagina ranging from 100 to 960,000 organisms per ml.

The present paper deals with the numbers of bacteria commonly encountered in bull semen and the relationship between the average numbers of bacteria in semen and the fertility level of bulls used for routine artificial breeding. A study of the incidence of specific types of bacteria in bull semen and their relation to fertility also has been completed and will be reported in a subsequent publication.

METHODS

The semen samples used in these studies were collected by means of the artificial vagina from Guernsey, Holstein and Jersey bulls located at the Western

Received for publication January 21, 1949.

¹ Authorized for publication January 18, 1949, as paper no. 1502 in the journal series of the Pennsylvania Agricultural Experiment Station.

Pennsylvania Artificial Breeding Cooperative, Clarion.² Routine collection techniques were followed in obtaining the samples and with few exceptions a different sterile artificial vagina was employed for the collection of each ejaculate. Usually two or more ejaculates were collected in succession from the same bull on each collection day. Occasionally when the first ejaculate was of sufficient volume and of satisfactory quality for insemination purposes, additional ejaculates were not collected. Although strict asepsis was not practiced in the handling of the semen samples, reasonable precautions were taken to prevent excessive bacterial contamination. The rubber innerliners of the artificial vagina were disinfected by immersion in 70 per cent alcohol and were allowed to air-dry prior to use. All glassware contacted by the semen was sterilized previously by dry heat in an oven at not less than 170° C. for at least 1 hour.

Since it was impractical to conduct the routine bacterial examinations at the headquarters of the Cooperative it was necessary to ship the undiluted semen samples to the laboratory at State College, a distance of about 105 miles. Subsamples of 1.5 ml. were taken from each ejaculate immediately after collection and placed in test tubes. These samples were cooled gradually to about 5° C. and then packaged for shipment. The test tubes were placed in a refrigerated cardboard shipping carton (12) and shipment then was made by special delivery parcel post. Upon arrival at the laboratory the temperature of each shipment was determined by inserting a precooled thermometer into a tube of water placed alongside the semen samples at the time of packaging.

The bacteriological examination was initiated within 1 hour after the samples arrived at the laboratory. The examination consisted of the inoculation of veal infusion agar, containing 4 per cent defibrinated bovine blood, with suitable dilutions of semen to determine the approximate numbers of living bacteria per ml. of semen. Sterile distilled water was used as the dilution medium and dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 were employed to obtain countable plates. In the early phases of the study the inoculated media were incubated at 37° C. for both 48 and 96 hours. Based on the results reported below, incubation for 48 hours was discontinued and all subsequent counts were made after 96 hours of incubation. As suggested by the American Public Health Association (6) all plate counts were adjusted to the first two significant figures and represent the number of colonies of bacteria that would have developed on the agar if an entire ml. of semen had been used.

RESULTS

Effect of short time, low temperature storage upon growth of bacteria in bull semen. Since shipment of the semen samples required about 24 to 32 hours at a temperature of approximately 5° C., it was necessary to conduct a preliminary study to determine the effect of short time, low temperature storage on the bacterial plate count of undiluted semen. Portions of four ejaculates from three bulls of the College dairy herd were plated within 30 minutes after collection and

² The authors wish to thank G. W. Thompson, Manager, for his valuable assistance in this study.

after 4, 12 and 32 hours of storage at 5° C. There were no significant differences in the plate counts of semen stored under these conditions.

Further studies were conducted to ascertain the reliability of plate counts of semen samples packaged and shipped by the Cooperative in the manner previously described. Plate counts on portions of seven ejaculates of semen plated immediately after collection at the Cooperative averaged 100,000 organisms per ml. Other portions of the same ejaculates plated at the College following routine shipment averaged 130,000 per ml. As a result of the slight difference obtained it is believed that the following data are representative of the bacterial content of freshly collected semen.

Effect of incubation time upon plate counts. In order to test the reliability of counts obtained following incubation for 48 and 96 hours at 37° C., a series of plates representing 54 ejaculates from 21 bulls were counted after 48 hours of incubation and recounted after a total incubation period of 96 hours. The average plate counts increased from 53,000 to 92,000 bacteria per ml. as the result of the additional 48 hours of incubation. Although increases were noted in the average plate counts of semen from all bulls after 96 hours of incubation, average plate counts of semen from only 4 of the 21 bulls showed an increase of more than five times the counts obtained after 48 hours. Bacterial growth obtained during the additional 48-hour incubation period consisted essentially of slow-growing diphtheroids apparently common to nearly all samples of bull semen. Similar observations were reported by Gunsalus *et al.* (10), who selected an incubation period of 96 hours to facilitate counting when slow-growing diphtheroids were present. Due to the relatively slight increases in plate counts, it is believed for the purposes of this study that the 48-hour counts are equally as significant as the 96-hour counts. However, the longer period of incubation is somewhat more reliable and an incubation period of 96 hours now is routine at this station for studies of this nature.

Bacterial plate counts of bull semen. Information on the numbers of bacteria commonly encountered in semen from bulls used in artificial breeding was obtained from plate counts of 202 ejaculates from 36 bulls. With the exception of six recently acquired bulls, none of them had been used in natural service for 6 months prior to the initiation of these studies. Table 1 shows that the arithmetic mean plate count of the 202 ejaculates was 200,000 organisms per ml. An extremely wide range of from less than 100 to more than 3 million per ml. was found. Marked differences were noted between samples from different bulls and between various ejaculates from the same bull. As indicated by the wide range of the average counts, a maximum difference of about 3,000-fold was observed between bulls. Differences between ejaculates from the same bull were as great as 2,000-fold.

Comparison of plate counts of first and second ejaculates. It has been reported (15, 16) that when several ejaculates are taken in succession, there are fewer bacteria in second than first ejaculates. In the present study the average plate count of 91 first ejaculates from 32 bulls was 220,000 per ml. as compared

to an average count of 130,000 per ml. for the same number of second ejaculates. In the case of 11 of the 32 bulls, the average counts of second ejaculates were greater than those of first ejaculates. The differences between first and second ejaculates were neither sufficiently large nor consistent enough to be considered significant.

Relationship between the plate counts of semen and the fertility level of bulls.

Fertility data for 33 bulls were collected for the period from July 1 to November

TABLE 1

Bacterial plate counts of semen from 36 bulls used in artificial breeding

Bull	No. of ejaculates	Plate count/ml.	
		Av.	Range
G- 1	4	1,200,000	400,000-2,300,000
G- 2	5	910,000	160,000-3,300,000
G- 3	6	20,000	2,200- 64,000
G- 4	9	19,000	75- 120,000
G- 5	15	320,000	2,200-1,000,000
G- 6	16	260,000	65-2,600,000
G- 7	4	7,900	2,600- 16,000
G- 8	2	3,900	2,600- 5,300
G- 9	3	1,500,000	1,000,000-2,100,000
G-10	2	4,900	50- 9,800
G-11	4	19,000	190- 50,000
G-12	5	74,000	550- 280,000
G-13	12	6,700	650- 36,000
G-14	9	36,000	600- 800,000
G-15	4	9,300	900- 120,000
G-16	2	56,000	6,600- 12,000
G-17	12	54,000	320- 270,000
H- 1	2	110,000	26,000- 190,000
H- 2	7	290,000	450-1,900,000
H- 3	5	60,000	1,100- 270,000
H- 4	4	49,000	9,100- 140,000
H- 5	2	2,600	1,200- 4,100
H- 6	1	570,000	
H- 7	4	300,000	2,100- 900,000
H- 8	1	19,000	
H- 9	2	600	100- 1,200
H-10	18	240,000	180-2,000,000
H-11	2	13,000	9,800- 17,000
H-12	2	3,400	2,200- 4,700
H-13	4	79,000	4,400- 200,000
H-14	14	140,000	4,700-1,300,000
H-15	8	46,000	640- 160,000
H-16	3	12,000	1,400- 2,600
J- 1	1	170,000	
J- 2	6	6,300	250- 210,000
J- 3	2	360,000	80,000- 650,000
Summary	202	200,000	50-3,300,000

1, 1947, based on the percentages of first and second service cows which did not return to service 90 to 120 days following the last insemination. The 33 bulls represented various levels of fertility as shown by the wide range of from 34 to 82 per cent 90- to 120-day non-returns. While the fertility data represent all ejaculates used for inseminations during the 4-month period, a few of the ejaculates were not available for bacterial examination.

The bulls were grouped on the basis of their general level of fertility and table 2 shows the averages as well as the ranges of the plate counts at the various fertility levels. Note that the lowest average plate count is associated with the low level of fertility; conversely, the highest average plate count was obtained from bulls at the high level of fertility. However, the differences appeared to be of insufficient magnitude to indicate any significant relationship between fer-

TABLE 2
Relationship of the bacterial plate count of semen to level of fertility

Level of fertility	No. of bulls	No. of ejaculates	Plate count/ml.	
			Av.	Range
High (66-82)*	10	47	290,000	200-3,300,000
Medium (56-65)	12	47	200,000	65-2,600,000
Low (34-55)	11	93	140,000	75-2,000,000
All levels	33	187	190,000	65-3,300,000

* Per cent 90- to 120-day non-returns.

tility and the plate count of semen. Nevertheless, the possibility still exists that the fertilizing capacity of any particular sample of semen may be affected by the number of bacteria present.

Relationship between the plate counts of semen and the age of bulls. A comparison of the plate counts of the semen samples from bulls of various ages was made and the data are presented in table 3. The 36 bulls were divided into five groups based on their ages as of July 1, 1947. Note that the lowest numbers of bacteria were found in semen from the bulls in the 6- to 7- and 8- to 9-year-old

TABLE 3
Relationship of the bacterial plate count of semen to age of bulls

Age (yr.)	No. of bulls	No. of ejaculates	Plate count/ml.	
			Av.	Range
1-3	10	35	220,000	75-3,000,000
4-5	13	75	240,000	50-3,300,000
6-7	6	40	84,000	640-1,300,000
8-9	4	29	210,000	65-2,600,000
10 and over	3	23	400,000	2,200-2,300,000
All ages	36	202	200,000	50-3,300,000

age groups, while the greatest numbers of bacteria were found in semen from the oldest group of bulls. However, the average count for the latter group was influenced markedly by the large numbers of bacteria contained in semen from one bull. The average plate count of the four ejaculates from this bull was 1,200,000 per ml. Since differences in plate counts of semen between age groups were not marked, there appears to be no important relationship between the age of bulls and the average plate count of semen.

SUMMARY

1. Wide variations were found in the bacterial plate counts of semen from various bulls and in the counts of semen collected at different times from the same animal. The plate count on 202 ejaculates from 36 bulls ranged from less than 100 to more than 3 million organisms per ml., with an average of 200,000 per ml.

2. No significant differences were observed in the plate counts of first and second ejaculates collected in succession from the same bull. Plate counts for 91 paired ejaculates showed 220,000 bacteria per ml. for first ejaculates and 130,000 per ml. for second ejaculates.

3. There was no apparent relationship between the average plate counts of semen and the general fertility level of bulls.

4. There was no important relationship between the age of bulls and the average plate count of semen.

REFERENCES

- (1) ALMQUIST, J. O. The Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. (Abs.) *J. Dairy Sci.*, 31: 681. 1948.
- (2) ALMQUIST, J. O., GLANTZ, P. J., AND SHAFFER, H. E. The Effect of a Combination of Penicillin and Streptomycin upon the Livability and Bacterial Content of Bovine Semen. *J. Dairy Sci.*, 32: 183-190. 1949.
- (3) ALMQUIST, J. O., GLANTZ, P. J., AND THORP, W. T. S. The Effect of Streptomycin upon the Livability and Bacterial Content of Bovine Semen. *J. Dairy Sci.*, 31: 501-507. 1948.
- (4) ALMQUIST, J. O., THORP, W. T. S., AND KNOTT, C. B. The Effect of Penicillin upon the Livability, Fertility and Bacterial Content of Bull Semen. *J. Animal Sci.*, 5: 400. 1946.
- (5) ALMQUIST, J. O., THORP, W. T. S., AND KNOTT, C. B. The Effect of Penicillin upon the Livability, Glycolysis and Bacterial Content of Bovine Semen. *J. Dairy Sci.*, 31: 11-19. 1948.
- (6) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 8th ed. 1941.
- (7) CAPOALE, G. Sul Contenuto Batterico dello Sperma Bovino Raccolto col Metodo della Vagina Artificiale. *Fecond. Artif. (Milano)*, 3: 7-11. 1941.
- (8) FOOTE, R. H., AND SALISBURY, G. W. The Effect of Pyridium, Penicillin, Furacin and Phenoxethol upon the Livability of Spermatozoa and upon the Control of Bacteria in Diluted Bull Semen. *J. Dairy Sci.*, 31: 763-768. 1948.
- (9) FOOTE, R. H., AND SALISBURY, G. W. The Effect of Sulfonamides upon the Livability of Spermatozoa and upon the Control of Bacteria in Diluted Bull Semen. *J. Dairy Sci.*, 31: 769-778. 1948.
- (10) GUNSALUS, I. C., SALISBURY, G. W., AND WILLETT, E. L. The Bacteriology of Bull Semen. *J. Dairy Sci.*, 24: 911-919. 1941.
- (11) KNOTT, C. B., AND SALISBURY, G. W. The Effect of Sulfanilamide upon the Livability and Metabolism of Bovine Spermatozoa. *J. Dairy Sci.*, 29: 285-291. 1946.
- (12) PERRY, E. J. The Artificial Insemination of Farm Animals. Rutgers Univ. Press, New Brunswick, N. J. Revised ed. 1948.
- (13) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (14) Report of the Chief of the Bureau of Animal Industry, U. S. Dept. Agr. P. 5. 1942.
- (15) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (16) SALISBURY, G. W., WILLETT, E. L., AND GUNSALUS, I. C. Some Problems in Bull Semen Storage. *Proc. Am. Soc. Animal Production*, 32: 210-212. 1939.

THE EFFECT OF FEEDING ALFALFA HAY CONTAINING DDT RESIDUE ON THE DDT CONTENT OF COW'S MILK^{1,2}

J. B. SHEPHERD AND L. A. MOORE, *Bureau of Dairy Industry* AND R. H. CARTER AND
F. W. POOS, *Bureau of Entomology and Plant Quarantine, Agricultural Research
Administration, U. S. D. A.*

Insecticides containing DDT now are being used on various crops grown as feed for milking cows. The relationship between the amount of DDT residue on the crop when fed, or the amount of DDT ingested by the cows, and the amount of DDT that may appear in the milk is not too well known. It is possible that enough DDT may be secreted in the milk to make it detrimental to consumers, especially if consumption of such milk is continued over a long period of time, since Kunze *et al.* (3) have reported that as little as 5 p.p.m. of DDT in the diet of the rat for 4 to 6 months will produce histopathological alterations of the liver.

Carter *et al.* (1) fed pea vine silage to milking cows at the rate of 3 lb. per 100 lb. of body weight. The silage contained 2.7 to 5.4 γ of DDT per g. on a fresh basis and 7.7 to 18.7 γ on a dry weight basis. The daily intake of DDT per cow was approximately 44 to 88 mg. The DDT content of the milk was less than 0.5 γ per g.

Wilson *et al.* (5) found 15 γ of DDT per g. in the milk from cows fed pea vine silage that provided an intake of about 1.5 g. of DDT per day per 1,000 lb. of body weight. These same investigators also found 44 γ per g. in the milk from a cow that received 24 g. of DDT per day.

This report gives the results of recent studies showing the concentration of DDT in the milk from cows fed alfalfa hay that had been treated with DDT under field conditions.

EXPERIMENTAL PROCEDURE

In August, 1947, a field of alfalfa, from which the third cutting was to be taken, was treated with different amounts of DDT by means of an aerosol machine. Part of the field was treated with 0.6 lb. of DDT per acre, the rate usually recommended for control of the potato leafhopper, and harvested 20 days later. The hay from this part of the field was designated as "Light DDT Hay." Another part of the field was treated with 2.4 lb. of DDT per acre, about four times the amount required for control of the leafhopper, and harvested 14 days later. This hay was designated as "Heavy DDT Hay." Both lots of hay were harvested in the quarter- to half-bloom stage, but both lots were of poor quality because they were rained on in the field and had to be cured on a barn hay-finisher.

The Heavy DDT Hay was fed to three cows (two Holsteins and a crossbred cow). In addition to the hay, all three cows received corn silage at the rate

Received for publication January 22, 1949.

¹ This work was supported in part with funds from the Agricultural Research and Marketing Act of 1946.

² Acknowledgement is made of assistance in the chemical work by H. D. Mann, P. E. Hubanks and E. C. Eckenrode, Bureau of Entomology and Plant Quarantine.

of 2 lb. per 100 lb. live weight daily, along with enough grain for normal production. The two Holstein cows received the DDT hay at the rate of 1 lb. per 100 lb. live weight daily. One Holstein (no. 1638) received the hay for 34 days before she calved and for 128 days afterwards, after which the hay was discontinued and she was turned on pasture. The other Holstein (no. 1666) had been fresh for 32 days when the DDT hay first was added to her ration; she received this hay for 111 days and thereafter received untreated hay. The crossbred cow (X-47) received the DDT hay at the rate of 0.5 lb. per 100 lb. live weight daily. She had been fresh for 17 days before the hay was added to her ration; she received the DDT hay for 110 days and then was turned on pasture.

The Light DDT Hay was fed to one crossbred cow (X-16). It was fed at the rate of 1.5 lb. per 100 lb. of live weight daily, along with corn silage at the same rate and enough grain for normal production. This cow had been fresh for 32 days when the DDT hay was substituted for untreated hay in her ration. She received the DDT hay for 98 days and then was turned on pasture.

The amount of all feed fed and refused was weighed accurately each day. A small portion of the treated hays was saved each day and composite samples were analyzed for DDT each month. Accurate records were kept of the daily milk production. A 2-day composite sample of milk was saved every 10-day period and analyzed for DDT. Because of the poor quality of the hay, the cows did not consume the quantities desired. However, since accurate weights were kept of the amount of hay fed and refused, the actual intake of hay was determined readily and the approximate DDT intake thus could be calculated.

The amount of DDT on the hay was calculated from the determinations of total organic chlorine in the residue, following the procedure of Carter and Hubanks (2). Approximately 300- to 500-g. samples of hay were extracted with benzene for 30 minutes in a tumbling apparatus. Aliquots of the benzene solution then were evaporated on the steam bath, and the residue was taken up in isopropanol and refluxed with metallic sodium for 1 hr. The chloride in solution then was determined by titration with standard silver nitrate, using an electro-metric titrimeter.

The amount of DDT in the milk was determined by the colorimetric method of Schechter *et al.* (4), as modified for use with milk samples. Samples of 50 ml. were extracted with a mixture of 75 per cent ethyl ether and 25 per cent petroleum ether. This ether extract then was evaporated and the butterfat containing the DDT was processed by the recommended procedure. It was found that two or more treatments with the sulfuric acid-sodium sulfate reagent, as well as two or more treatments with the concentrated sulfuric acid-fuming sulfuric acid reagent, were of value in the separation of the DDT from the butterfat.

RESULTS AND DISCUSSION

As shown in table 1, the colostrum milk from cow no. 1638 contained 14 γ of DDT per g. The maximum amount of DDT in the milk was 10.1 γ per g. This was equivalent to 259.1 γ per g. of butterfat, or 94,597 γ per lb. of butter containing 80.5 per cent butterfat. The average daily intake of DDT for the entire

feeding period was 553 mg., while the average daily output during the milking period was 165 mg. DDT was detectable in the milk for 160 days after the DDT-treated hay was removed from the ration.

As shown in table 2, cow no. 1666 began secreting DDT in her milk after 3 days on DDT hay, and the average for the first 10-day period was 3.2 γ of DDT

TABLE 1

Average daily intake of alfalfa hay and DDT for cow no. 1638 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(lb.)	(γ /g.)	(mg.)	(lb.)	(γ /g.)	(mg.)	(γ /g.)
11- 6-47 ^a	11.7	121.0	642.2	0.0			
11-16-47	10.1	121.0	554.3	0.0			
11-26-47	10.5	121.0	576.3	0.0			
12- 6-47 ^b	14.5	121.0	795.8		14.4 ^c		
12-16-47	13.2	121.0	724.5	56.4	8.9	227.7	222.6
12-26-47	12.4	121.0	680.6	57.8	10.1	264.8	259.1
1- 5-48	12.6	74.2	424.1	56.5	7.4	189.7	217.8
1-15-48	12.9	74.2	434.2	53.4	6.9	167.1	174.6
1-25-48	8.8	74.2	296.2	53.2	9.0	217.2	250.0
2- 4-48	8.2	134.5	500.3	50.6	6.7	153.8	186.2
2-14-48	10.0	134.5	610.1	48.4	6.8	149.3	186.1
2-24-48	8.9	134.5	543.0	46.4	4.0	84.2	105.3
3- 5-48	8.2	128.4	477.6	45.0	6.0	122.5	166.7
3-15-48	8.1	128.4	471.8	44.0	8.4	167.7	221.2
3-25-48	8.9	128.4	518.4	41.1	6.7	124.9	191.3
4- 4-48	10.4	128.4	605.7	45.3	6.4	112.3	149.7
4-14-48 ^d				46.8	2.9	61.6	82.9
4-24-48				46.3	1.6	33.6	49.6
5- 4-48				44.1	0.9	18.0	24.3
5-14-48				40.7	0.5	9.2	13.3
5-24-48				41.8	0.3	5.7	9.0
6- 3-48				37.4	0.2	3.4	4.0
6-13-48				36.2	0.3	4.9	7.9
6-23-48				34.6	0.2	3.1	6.8
7- 3-48				37.3	0.2	3.4	5.7
7-13-48				29.0	0.2	2.6	5.3
7-23-48				30.4	0.3	4.1	8.5
8- 2-48				31.1	0.1	1.4	2.9
8-12-48				32.6	0.2	3.0	5.1
8-22-48				23.9	0.1	1.1	2.3
9- 1-48				23.4	0.2	2.1	5.5
9-11-48				24.1	0.1	1.1	3.0
9-21-48				19.2	0.1	0.9	2.6
10- 1-48				22.2	Trace		
10-11-48				16.1	0.0		

^a Started on Heavy DDT Hay November 4, 1947, at rate of 1 lb. per 100 lb. of live weight.

^b Calved December 8, 1947. Average weight 1,300 lb.

^c In colostrum milk.

^d DDT hay discontinued and cow turned on pasture 0.5 day per d. for 3 d.; on pasture continuously after April 17, 1948.

per g. of milk. The highest value for any 10-day period was 9.7 γ per g. of milk. The average daily intake of DDT for the 111-day feeding period was 727 mg. and the average daily output was 136 mg. DDT was detectable in the milk for 170 days after the DDT hay was removed from the ration.

While the amount of DDT in the milk of both cows (no. 1666 and 1638) was about the same at the time the treated hay was removed from the ration, the concentration of DDT in the milk of no. 1638 decreased more rapidly than that in the milk of no. 1666, although the milk from both cows showed the presence of DDT for about the same length of time. Cow no. 1638 was turned on pasture, whereas cow no. 1666 was kept on dry feed; this might account for the difference in rate of decrease in DDT concentration.

TABLE 2

Average daily intake of alfalfa hay and DDT for cow no. 1666 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(lb.)	($\gamma/g.$)	(mg.)	(lb.)	($\gamma/g.$)	(mg.)	($\gamma/g.$)
12-26-47 ^a	11.7	121.0	642.2	58.4	3.2	84.8	69.6
1- 5-48	13.5	74.2	454.4	58.6	3.8	101.0	86.4
1-15-48	14.8	74.2	498.1	57.2	4.9	127.1	111.4
1-25-48	13.5	74.2	454.4	55.8	8.7	220.2	185.0
2- 4-48	14.5	134.5	884.6	52.4	6.7	159.2	152.2
2-14-48	14.8	134.5	902.9	48.8	6.3	139.5	146.5
2-24-48	14.7	134.5	896.8	43.1	4.5	88.0	99.0
3- 5-48	14.6	128.4	850.3	42.0	7.9	150.5	175.6
3-15-48	13.4	128.4	780.4	41.3	9.7	181.7	215.5
3-25-48	13.8	128.4	803.7	36.5	6.9	114.2	148.3
4- 4-48	14.3	128.4	832.9	38.1	7.2	124.4	159.9
4-14-48 ^b				30.4	6.2	85.5	118.1
4-24-48				34.4	2.4	37.4	51.0
5- 4-48				30.3	2.4	33.1	50.2
5-14-48				28.8	1.2	15.7	25.3
5-24-48				30.9	0.5	7.0	10.2
6- 3-48				26.8	0.6	7.2	16.0
6-13-48				28.6	1.0	13.0	20.0
6-23-48				24.5	0.7	7.8	14.3
7- 3-48				24.4	0.4	4.4	7.0
7-13-48				24.2	0.3	3.3	6.4
7-23-48				26.6	0.5	6.0	10.2
8- 2-48				22.4	0.3	3.0	6.0
8-12-48				23.9	0.4	4.3	8.4
8-22-48				19.6	0.5	4.4	9.7
9- 1-48				22.3	0.4	1.0	2.1
9-11-48				22.4	0.3	3.0	6.2
9-21-48				21.4	0.3	2.9	6.5
10- 1-48				17.5	Trace		
10-11-48				16.5	Trace		

^a Calved November 24, 1947; started on Heavy DDT Hay on December 26, 1947, at rate of 1 lb. per 100 lb. of live weight. Av. weight 1,475 lb.

^b Changed to hay containing no DDT on April 15, 1948.

Cow X-47 (table 3) received a smaller allowance of Heavy DDT Hay for 110 days, and therefore a smaller intake of DDT, than the other two cows. She also showed a smaller quantity of DDT in her milk, the highest value being 3.0 γ per g. The average daily intake of DDT was 303 mg. and the average daily output was 51 mg. No DDT was detectable in the milk of this cow after she was on pasture for 40 days.

TABLE 3

Average daily intake of alfalfa hay and DDT for cow X-17 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(lb.)	($\gamma/g.$)	(mg.)	(lb.)	($\gamma/g.$)	(mg.)	($\gamma/g.$)
12-26-47 ^a	6.1	121.0	334.8	55.2	1.4	35.0	23.7
1- 5-48	4.7	74.2	157.4	57.1	0.4	10.4	7.4
1-15-48	5.7	74.2	188.8	60.3	2.3	62.9	47.9
1-25-48	4.6	74.2	154.8	55.3	2.8	70.3	58.4
2- 4-48	5.7	134.5	347.8	54.8	3.2	79.5	65.3
2-14-48	6.0	134.5	366.1	50.7	2.7	62.1	52.9
2-24-48	6.0	134.5	366.1	48.2	1.3	28.4	28.9
3- 5-48	5.9	128.4	343.6	46.4	1.7	35.8	31.2
3-15-48	6.0	128.4	349.4	46.3	2.9	61.0	60.5
3-25-48	5.9	128.4	343.6	44.5	3.0	60.6	65.2
4- 4-48	6.1	128.4	355.3	45.1	2.5	51.1	50.5
4-14-48 ^b				45.8	1.3	27.0	26.0
4-24-48				43.6	0.4	7.9	8.0
5- 4-48				35.1	0.0	"	
5-14-48				32.7	0.1	1.4	2.0
5-24-48				34.3	0.0		
6- 3-48				30.1	0.0		
6-13-48				25.3			

^a Calved December 9, 1947, started on Heavy DDT Hay, at the rate of 0.5 lb. per 100 lb. of live weight on December 26, 1947. Average weight 1,175 lb.

^b Turned on pasture April 14, 1948, and DDT hay discontinued.

TABLE 4

Average daily intake of alfalfa hay and DDT for cow X-16 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(lb.)	($\gamma/g.$)	(mg.)	(lb.)	($\gamma/g.$)	(mg.)	($\gamma/g.$)
12-26-47 ^a	8.8	4.4	17.6	26.9	0.0	0.0	
1- 5-48	12.1	11.2	61.5	27.9	0.2	2.5	4.7
1-15-48	14.2	11.2	72.1	27.0	0.6	7.3	14.2
1-25-48	14.6	11.2	74.2	26.9	0.4	4.9	9.3
2- 4-48	14.7	13.7	91.4	25.5	0.2	2.3	4.4
2-14-48	14.5	13.7	90.1	23.3	0.3	3.2	5.9
2-24-48	14.7	13.7	91.4	22.1	0.55	5.5	12.8
3- 5-48	14.9	30.5	197.0	21.6	0.8	7.8	19.0
3-15-48	14.9	30.5	197.0	21.1	0.9	8.6	21.1
3-25-48	14.2	30.5	196.5	20.9	0.6	5.7	14.3
4- 4-48 ^b				22.3	0.3	3.0	6.7
4-14-48				24.2	0.4	4.4	8.4
4-24-48				19.1	0.1	0.9	2.3
5- 4-48				20.5	0.0	0.0	
5-14-48				17.7	0.0	0.0	
5-24-48				18.0	0.0	0.0	
6- 3-48				14.6	0.0		

^a Calved November 24, 1947, started on Light DDT Hay December 26, 1947, at rate of 1.5 lb. per 100 lb. of live weight.

^b Turned on pasture April 2, 1948; DDT hay discontinued.

Cow X-16 (table 4) had a much smaller DDT intake than the three cows that were fed the Heavy DDT Hay, and also showed the lowest concentration of DDT in the milk. The highest concentration of DDT in her milk was 0.9 γ per g. The average daily intake of DDT was 109 mg. and the average daily output was 4.8 mg. DDT was no longer detectable in her milk 30 days after the DDT hay was removed from her ration and she was turned on pasture.

A cursory examination of the data indicated the possibility of a relationship between the DDT intake, the DDT concentration in the milk and the total output of DDT in the milk. Table 5 shows that the four cows in this experiment secreted from 5 to 30 per cent of the DDT intake into the milk. Because of differences in the length of the feeding period and in total milk production, no good correlation between DDT intake and output could be expected. However, there was some correlation between the intake of DDT and the concentration of DDT in the milk. Similar data reported by Carter *et al.* (1) and Wilson

TABLE 5
Average daily intake of DDT from the feed and output in milk

Cow no.	Interval DDT hay was fed	Av. daily intake	Av. concentration in milk	Av. daily output in milk	% intake in milk	
					During DDT feeding	After DDT feeding
	(d.)	(mg.)	(γ /g.)	(mg.)	(%)	(%)
<i>Present experiment</i>						
1666	111	727	6.4	136	18.6	1.8
1638	162	553	7.3	165	29.8 ^a	3.0
X-47	110	303	2.2	51	16.8	1.1
X-16	98	109	0.5	5	4.8	0.6
<i>Other experiments</i>						
Carter (1)	53	44-88 ^b	0.5			
Wilson (4)	141	1500 ^b	15.0			
Wilson (4)	150	24000 ^c	44.0			

^a The output in the milk was 22.4% when the total intake and output are considered, since this cow received DDT during the previous dry period.

^b Fed pea vine silage.

^c Fed crystalline DDT.

et al. (5), which also are shown in table 5, seem to show a similar relationship. In order to get a better correlation between these various factors, however, the hay samples in the present experiment should have been analyzed more frequently during the feeding period.

Considerable DDT appeared in the milk from the three cows fed hay from a field treated with 2.4 lb. of DDT per acre. This rate of applying DDT was at least four times as heavy as normally would be required for control of the potato leafhopper in alfalfa. A much smaller quantity of DDT appeared in the milk from the cow that received the hay from a field that was treated with only 0.6 lb. of DDT per acre, about the recommended rate of application. The DDT appeared in the milk after the cows were on DDT for only a few days, and in one case DDT was present after only 3 days on DDT hay.

The fact that these hays were damaged rather badly by rain may have had considerable effect on the results. Hays similarly treated but cured without rain might have been consumed in larger quantities, thereby increasing the intake of DDT and the output of DDT in the milk. These results indicate that care should be taken not to apply more DDT than actually is needed for insect control; otherwise, if the crop is fed to milking cows, considerable DDT will appear in the milk.

Other studies made in connection with this experiment indicate that weathering is helpful in reducing the amount of DDT on the forage at cutting time; therefore, it seems probable that if minimum effective dosages are applied about midway in the development of the crop at least 21 to 25 days prior to cutting, the DDT residue hazard will be reduced greatly.

SUMMARY

1. Alfalfa treated with 2.4 lb. of DDT per acre, in the form of an aerosol, and fed to cows at the rate of 1 lb. of hay per day per 100 lb. of body weight produced milk containing up to 10.1 γ of DDT per g. or 259.1 γ per g. of butterfat. The daily intake of DDT was as high as 903 mg. and the output in the milk was as high as 265 mg.

2. Alfalfa treated with 0.6 lb. of DDT per acre and fed to cows at the rate of 1.5 lb. of hay per 100 lb. of body weight produced milk containing up to 0.9 γ of DDT per g.

3. The output of DDT in the milk varied from 5 to 30 per cent of the intake. The DDT appeared in the milk after a very few days of feeding, and in one case was present in appreciable quantities after 3 days of feeding.

4. After the feeding of DDT hay was discontinued, DDT was detected in the milk for 160 to 170 days when large quantities of DDT had been fed and for only 30 to 40 days when small quantities had been fed.

REFERENCES

- (1) CARTER, R. H., HUBANKS, P. E., MANN, H. D., SMITH, F. F., PIQUETT, P. G., SHAW, J. C., AND DITMAN, L. P. The D.D.T. Content of Milk from Cows Fed Pea Vine Silage Containing D.D.T. Residues. *J. Econ. Entomol.*, **42**: 119. 1949.
- (2) CARTER, R. H., AND HUBANKS, P. E. Determination of D.D.T. Deposits on Fruits, Vegetables and Forage Crops. *J. Assoc. Offic. Agr. Chemists*, **29**: 112. 1946.
- (3) KUNZE, FRIEDA, NELSON, A. A., FITSHUGH, O. G., AND LAUG, E. P. Storage of DDT in the Fat of the Rat. *Federation Proc.*, **8**, 1: 311. 1949.
- (4) SCHECHTER, M. S., POGORELSKIN, M. A., AND HALLER, H. L. Colorimetric Determination of D.D.T. in Milk and Fatty Materials. *Anal. Chem.*, **19**: 51. 1947.
- (5) WILSON, H. F., ALLEN, N. N., BOHSTEDT, G., BETHEIL, J., AND LARDY, H. A. Feeding Experiments with D.D.T.-treated Pea Vine Silage with Special Reference to Dairy Cows, Sheep and Laboratory Animals. *J. Econ. Entomol.*, **39**: 801. 1946.

LOSS OF REDUCED ASCORBIC ACID FROM LACTOSE- ENRICHED MILK¹

ARTHUR D. HOLMES

*Massachusetts Agricultural Experiment Station,
Amherst*

In a study of the rate of disappearance of reduced ascorbic acid from samples of commercial milk, Hand (4) observed a decrease from 19 to 7 mg. per l. for milk stored 6 days at 1° C. Kon and Watson (9), Holmes and Jones (6), Buruiana (1), Krauss (10), Diemair and Fresenius (2) and others have noted the rapid loss of ascorbic acid from milk, particularly when the milk was exposed to light. Holmes and Jones (8) determined the permanency of synthetic ascorbic acid added to milk and found the same trend, but a less rapid loss of reduced ascorbic acid than that reported by Gunsalus and Hand (3). However, in a study of the stability of reduced ascorbic acid in mares' milk (mares' milk may contain from five to ten times as much ascorbic acid as cows' milk), Holmes and Jones (7) obtained data which show that the rate of loss of reduced ascorbic acid from mares' milk was only a fraction of the rate of loss from cows' milk when both were stored in commercial glass milk bottles in the dark at 10° C. Naturally, a question arose concerning the factor or factors that caused the reduced ascorbic acid of mares' milk to be more stable than that of cows' milk. Linton (11), Hildebrandt (5) and others have reported that mares' milk contains much more lactose than cows' milk. Since for decades it has been a common practice in the preparation of modified milk formulas for infant feeding to add lactose to cows' milk to approximate the quantity in human milk, it seemed desirable to determine whether the addition of lactose to cows' milk would decrease the rate of disappearance of reduced ascorbic acid from cows' milk to that observed for mares' milk.

EXPERIMENTAL PROCEDURE

In this study the samples of commercial pasteurized cows' milk were stored in the dark at 10° C. in a home-size electric refrigerator. Each week when the samples were prepared, all the milk was mixed thoroughly in a single container to insure identical milk for the three series of samples. Fifteen g. per l. of analytical reagent grade of *alpha*-lactose were added to one series of samples, 30 g. per l. were added to a second series and no lactose was added to a third series of samples which served as controls. For 15 weeks in the period from April to August, one sample of each series was prepared on Monday morning and each sample was assayed for its reduced ascorbic acid content daily from Monday to Friday, inclusive. During the period of observation the samples were stored in commercial, flint, quart milk bottles. As the aliquots were withdrawn for assay the volume of milk in the bottles decreased and the volume of supernatant atmos-

Received for publication January 24, 1949.

¹ Contribution no. 695, Massachusetts Agricultural Experiment Station.

phere increased, which is the normal condition for the household use of milk. The reduced ascorbic acid content of the various milk samples was determined by a modification of Sharp's (12) rapid method. Twenty-five ml. of a mixture of 8 per cent acetic acid and 3 per cent metaphosphoric acid was added to 25 ml. of milk and the mixture was diluted with 25 ml. of distilled water and titrated with a standardized solution of sodium 2,6-dichlorbenzenoneindolphenol.

RESULTS AND DISCUSSION

The initial reduced ascorbic acid content of the pasteurized commercial milk used in this study varied from 9.0 mg. per l. to 17.8 mg. and averaged 14.7 mg. per l. After storage for 1 day the amount of reduced ascorbic acid in the 15 samples of each series averaged 8.6 mg. per l. for the controls, 8.3 mg. for the milk with 15 g. of lactose per l. added and 8.1 mg. for that with 30 g. of lactose added. At the end of storage for 2 days, the average values were 5.5 mg. per l. for the control samples, 4.8 mg. for the milk with 15 g. of lactose per l. added and 4.9 mg. for the milk with 30 g. of lactose added. When 3 days had elapsed, the average amounts of reduced ascorbic acid present in the different series of samples were 4.4 mg., 4.0 mg. and 4.1 mg., respectively. At the end of the 96-hour experimental period the same amount of reduced ascorbic acid was found in each of the three series of samples, i.e., 3.8 mg. per l. Judged by the data assembled here, the addition of reagent grade of *alpha*-lactose did not inhibit either the rate or amount of loss of reduced ascorbic acid from commercial cows' milk stored in darkness at 10° C. for 96 hours, a period comparable to the length of time that milk may be stored in partially-filled containers in the home.

These data indicate that the high lactose content of mares' milk is not the primary factor in the slower disappearance of reduced ascorbic acid from mares' than from cows' milk. However, it should be recognized that the lactose in mares' milk in excess of that in cows' milk may not be in the same form as the reagent grade used in this study and hence may react in a manner different from the lactose that was added to the cows' milk.

The rate of decrease in the amount of reduced ascorbic acid present in the different series of milk samples included in this study was slightly higher but very similar to that reported by Hand (4), i.e., 19.0 mg. on the first day, 15.6 mg. on the second day, 10.9 mg. the fourth day and 7.1 mg. of reduced ascorbic acid per l. of milk on the sixth day after milking. Gunsalus and Hand (3) also noted a decrease of reduced ascorbic acid in raw cows' milk during storage for 6 days of from 14.9 mg. to 1.7 mg. per l. or an average daily loss of over 14 per cent as compared with an average daily loss in this study of over 18 per cent. Possibly the amount of ascorbic acid in milk may influence the rate of disappearance of reduced ascorbic acid from milk, for when Holmes and Jones (8) added 75 mg. per l. of ascorbic acid to raw milk, the loss was 7 per cent per day for a 10-day storage, and when 150 mg. per l. was added, the loss for the 10-day period was 3 per cent per day. Both of these losses were less than the 10 per cent loss reported by Hand (4) or the 14.7 per cent loss found by Gunsalus and Hand (3). However, in

this study no significant difference was noted in the loss of ascorbic acid from the control samples and those enriched with 15 or 30 g. of lactose per l.

SUMMARY

There was no significant difference in the rate of loss of reduced ascorbic acid, during storage in darkness for 96 hours at 10° C. of a series of cows' milk samples to which 15 g. per l. of *alpha*-lactose had been added, a series of samples to which 30 g. per l. of lactose was added and a series of control samples. These results lead to the conclusion that, even though the lactose in mares' milk may be in a different form than that used in this study, the high lactose content of mares' milk is not the principal factor in causing the greater stability of reduced ascorbic acid in mares' milk than in cows' milk.

ACKNOWLEDGMENT

The author wishes to thank Mr. Elliott Greenwood of the Dairy Industries Department for supplying the milk used in this study.

REFERENCES

- (1) BURUANA, L. The Action of Sunlight on Milk. *Biochem. J.*, 31: 1452. 1937.
- (2) DIEMAIR, W., AND FRESENIUS, W. The Vitamin C Content of Milk. *Z. anal. Chem.*, 120: 313. 1940. (*C.A.*, 35: 1524. 1941.)
- (3) GUNSALUS, I. C., AND HAND, D. B. The Use of Bacteria in the Chemical Determination of Total Vitamin C. *J. Biol. Chem.*, 141: 853. 1941.
- (4) HAND, D. B. Reduced and Total Vitamin C in Milk. *J. Dairy Sci.*, 26: 7. 1943.
- (5) HILDEBRANDT, A. Analysis of Mares' Milk. *Milchzw. Zentr.*, 46: 273, 289, 305, 317. 1917. (*C. A.*, 12: 1480. 1918.)
- (6) HOLMES, A. D. AND JONES, C. P. Effect of Sunshine upon the Ascorbic Acid and Riboflavin Content of Milk. *J. Nutrition*, 29: 201. 1945.
- (7) HOLMES, A. D., AND JONES, C. P. Stability of Reduced Ascorbic Acid in Mares' Milk. *J. Nutrition*, 34: 113. 1947.
- (8) HOLMES, A. D., AND JONES, C. P. Permanency of Synthetic Ascorbic Acid Added to Milk. *J. Dairy Sci.*, 31: 99. 1948.
- (9) KON, S. K., AND WATSON, M. B. The Effect of Light on the Vitamin C of Milk. *Biochem. J.*, 30: 2273. 1936.
- (10) KRAUSS, W. E. Progress of Agricultural Research in Ohio. *Ohio Agr. Expt. Sta. Bull.* 617: 49. 1940.
- (11) LINTON, R. G. The Composition of Mares' Milk. *J. Agr. Sci.*, 21: 669. 1931.
- (12) SHARP, P. F. Rapid Method for the Quantitative Determination of Reduced Ascorbic Acid in Milk. *J. Dairy Sci.*, 21: 85. 1938.

THE RELATIONSHIP OF PRODUCTION OF HEIFERS MILKED PREPARTUM TO THE COMPOSITION OF COLOSTRUM¹

A. H. VANLANDINGHAM, C. E. WEAKLEY, JR., R. A. ACKERMAN,
AND GEORGE HYATT, JR.

West Virginia Agricultural Experiment Station, Morgantown

Many heifers and cows have badly swollen and congested udders before freshening. It is thought that such a condition makes it undesirable to put these animals on full feed and to try to bring them into full production until the swelling has subsided. Sometimes the udder becomes so distended that some believe that the gland may be injured permanently. Breeders frequently are concerned about the advisability of milking such animals before parturition.

Turner (5) was of the opinion that prepartum milking for a period of about 10 days has certain advantages. In all cases when cows were milked prepartum the udders were soft and pliable. The colostral character of the milk largely disappeared and the globulin content was reduced to that of normal milk at parturition. From the standpoint of the well-being of the calf, the initiation of milk secretion prior to parturition caused many of the calves to die at an early age. They seemed much more susceptible to *Bacillus (Escherichia) coli* infection and other calf diseases.

Keyes *et al.* (2) reported that 25 cows and heifers had been milked for a period of 2 to 16 days prepartum. They observed wide variations in the percentage total solids until the seventh day before parturition, when the solids content became more uniform and gradually approached that of normal milk at the time of parturition. The production of milk for the prepartum-milked cows started at about 1 lb. a day and increased to 25 lb. on the day of parturition. The calves had scours and showed signs of general inactivity, a condition which was corrected when 5 ml. of "carotone" (a carotene preparation) was fed daily for 7 days.

Turner (5) suggested that prepartum milking might reduce the incidence of milk fever by a gradual initiation of lactation, a suggestion that was not supported by recent work of Smith and Blosser (4). Eaton *et al.* (1) reported that the first milking after calving from cows which had not been milked prepartum contained approximately 5 times as much carotene and vitamin A, 3 to 4 times as much protein, 0.5 as much lactose, slightly greater amounts of fat and 1.25 times as much ash as the milk obtained from the cows milked prepartum. They concluded that prepartum milking materially alters the composition and lowers the nutritive value of the first milk secreted at the termination of pregnancy.

Because of the possibility of decreasing the inflammation and congestion of the udder at calving by prepartum milking and because of the adverse effect which

Received for publication February 2, 1949.

¹ Published with the approval of the director of the West Virginia Agricultural Experiment Station as scientific paper no. 403.

this practice might have on the nutrition of the calf, further study seemed warranted.

EXPERIMENTAL PROCEDURE

During the winter of 1947-1948, 16 first-calf Ayrshire heifers in the station herd were divided into two groups according to their expected freshening dates. It was planned that every other heifer was to be milked prepartum for a period of 2 weeks; however, some of the heifers calved before the expected dates of parturition. The heifers were milked prepartum with machines once daily until production increased to 2 lb., after which time they were milked twice daily and samples were taken for analysis. Accurate records of production were obtained

TABLE 1
The production and composition of colostrum on day of parturition

No. of heifer	Period milked prepartum	Production day before parturition	Total production before parturition	Composition of colostrum day of parturition		
				total N	non-casein N	non-casein N of total N
	(d.)	(lb.)	(lb.)	(g./100 ml.)	(g./100 ml.)	(%)
Group 1						
792	0	0	0	2.46	1.61	65.4
796	0	0	0	2.42	1.31	54.1
799	0	0	0	2.54	1.52	59.8
800	0	0	0	2.16	1.60	74.1
804	0	0	0	2.54	1.45	57.1
806	0	0	0	1.95	1.06	54.4
811	0	0	0	2.45	0.96	39.2
815	0	0	0	2.17	1.18	54.4
Av.	0	0	0	2.34	1.34	57.3
Group 2						
790	18	10.5	31	1.07	0.38	35.5
791	8	20.1	48	0.67	0.21	31.3
794	14	0.1	2	1.49	0.66	44.3
798	10	24.3	142	0.55	0.11	20.0
802	7	3.9	16	1.35	0.67	49.6
803	3	1.0	2	1.80	1.09	60.6
808	16	16.6	55	0.71	0.14	19.7
810	3	5.1	7	1.41	0.73	51.8
Av.	10	10.2	38	1.13	0.50	39.1

until the time of parturition. The calves remained with the dams for the first 3 days following parturition as was the usual practice. After parturition the udders were milked out twice daily and samples taken for analysis.

Samples of milk or colostrum were analyzed for total nitrogen by the Kjeldahl method. Casein was precipitated and non-casein nitrogen determined directly, whereas casein nitrogen was determined by difference (3).

RESULTS

Data presented in table 1 show the total nitrogen, non-casein nitrogen and the per cent non-casein nitrogen of the total nitrogen in the colostrum of the heifers in both groups on the day of parturition. For the heifers in group 2, which were milked prepartum, production on the day before parturition varied from

0.1 to 24.3 lb. Total production before parturition varied from 2 to 142 lb. The level of production on the day before parturition was dependent upon the extent that the heifers were stimulated into production. The number of days milked prepartum was not necessarily a determining factor. Heifer 794 was milked for 14 days and during this period produced only 2 lb. as compared with 798 which was premilked 10 days and produced 142 lb.

The total nitrogen, the non-casein nitrogen and the per cent non-casein nitrogen of the total nitrogen was much higher on the day of parturition for the heifers in group 1 than for those in group 2. Much greater variations existed in the composition of the milk for the heifers in group 2. The total nitrogen varied from 0.55 to 1.49 g. per 100 ml. and the non-casein nitrogen from 0.11 to 1.09 g. per 100 ml. The per cent non-casein nitrogen of the total nitrogen varied from 20.0 for heifer 798, which had produced a total of 142 lb. before parturition, to 60.6 per cent for heifer 803, which had been milked only 3 days prepartum and had produced only 2 lb. before parturition.

Total nitrogen, non-casein nitrogen and the per cent non-casein nitrogen of the total nitrogen are good criteria to indicate when the colostrum period is over and normal milk is being produced. Total nitrogen in the colostrum of the heifers in group 1 not milked prepartum decreased from an average of 2.34 on the day of parturition to 0.70 g. per 100 ml. on the fourth day after calving when normal milk was produced. During the same period the per cent non-casein nitrogen of the total nitrogen decreased from an average of 57.3 to 20.8.

Data presented in table 2 show the relationship between production and the nitrogen content of colostrum of four heifers in group 2 which were milked prepartum. Heifer 798 was milked 10 days before parturition. Production increased rather rapidly and 24.3 lb. were produced on the day before parturition. The total nitrogen and the per cent non-casein nitrogen decreased to the level of normal milk 3 days before parturition. Therefore this heifer's calf did not receive any true colostrum. Prepartum milking of heifer 808 was started 16 days before parturition; however, she did not produce as much as 1 lb. per day until the seventh day before calving, after which her production increased rapidly to 16.5 lb. on the day before calving. Changes in total nitrogen and non-casein nitrogen were very similar to that of 798, except that normal milk was produced only 1 day before calving. Again, this heifer's calf did not receive any true colostrum. Heifer 790 was milked 18 days prepartum. Production increased slowly until the third day before calving, at which time she produced about 5 lb. Production continued to increase so that 10.5 lb. were produced on the day before parturition. Milk of normal composition was produced on the second day after calving. Heifer 802 was milked 7 days prepartum. She produced only 3.9 lb. on the day before parturition. The total nitrogen and the per cent non-casein nitrogen after parturition were similar to those of the heifers in group 1 not milked prepartum.

DISCUSSION

In this study the total nitrogen, casein nitrogen and the non-casein nitrogen have been used to compare the composition of colostrum with that of normal milk.

Years ago Turner (5) presented data showing that colostrum was unusually rich in globulin and albumin and that the total protein was three to four times as high as in normal milk. Because of the large difference in nitrogen content of colostrum and normal milk and because of the ease in the determination of total nitrogen, this constituent is of strategic value in determining the rate of change from the production of colostrum to the production of normal milk. This is true for cows milked prepartum as well as cows milked postpartum.

The data presented show that prepartum milking affects the rate of change from the production of colostrum to that of normal milk as it affects the rate of production. The total nitrogen and the per cent non-casein nitrogen of the milk on the day of parturition were dependent upon the total amount of prepartum colostrum produced and the level of production at parturition. The composition of colostrum produced on the day of parturition was not related necessarily to the number of days heifers were milked prepartum. Some heifers were stimulated into production before parturition by prepartum milking, whereas others were not.

According to Turner (5), the initiation of milk secretion in late pregnancy and postpartum is due to the pituitary hormone lactogen. Following parturition, the secretion of milk is stimulated intensely by the rising secretion of the lactogenic hormone. While the presence of estrogen in increasing amounts just before parturition initiates the intense secretion of the lactogenic hormone, the stimulus of milking and the removal of milk are the factors that maintain the lactation process.

It seems reasonable to suspect that prepartum milking might cause a stimulation in the production of the lactogenic hormone, which in turn stimulates the secretion of the mammary gland. If this is true, it seems that in the case of some animals the manipulation of the udder and teats a few days prior to parturition causes the secretion of the lactogenic hormone, whereas, in the case of other animals, the lactogenic hormone is not secreted in appreciable amounts until after parturition.

As to the effect of prepartum milking on the condition of the udder following parturition and on the nutrition of the calf, more data are needed before any recommendation can be made. Of the eight calves dropped by the heifers in group 2 milked prepartum, only two failed to receive some colostrum. Each calf was given 25 ml. of cod liver oil on the day of birth to provide additional vitamins A and D. The calves were thrifty and made satisfactory growth.

SUMMARY AND CONCLUSIONS

During the fall and winter of 1947-1948, 16 first-lactation Ayrshire heifers were divided into two equal groups upon the basis of expected dates of parturition, every other animal being assigned to each group. Group 1 was handled in the usual way. The heifers in group 2 were milked for a period of 3 to 18 days with an average of 10 days before parturition.

The total production before calving for prepartum-milked heifers varied from 2 to 142 lb. The production on the day before parturition varied from 0.1 to 24.3 lb. with an average of 10.2 lb.

The total nitrogen and the non-casein nitrogen of the milk produced on the day of parturition for the heifers not milked prepartum averaged 2.34 and 1.34 g. per 100 ml., respectively, whereas the milk from the heifers milked prepartum averaged 1.13 and 0.50 g. per 100 ml., respectively. The non-casein nitrogen amounted to 57.3 per cent of the total nitrogen for the heifers not milked prepartum, but only 39.1 per cent for the heifers milked prepartum. The total nitrogen and the non-casein nitrogen in the milk of heifers prepartum-milked on the day of parturition were dependent on the level of production and the total amount produced before parturition.

Heifers producing appreciable quantities of prepartum colostrum produced normal-appearing milk at the time of parturition.

REFERENCES

- (1) EATON, H. D., SPIELMAN, A. A., JOHNSON, R. E., MATTERSON, I. D., AND SLATE, R. J. The Effect of Prepartum Milking on the Carotene and Vitamin A and Proximate Composition of Colostrum. *J. Dairy Sci.*, **31**: 685-686. 1948.
- (2) KEYES, E. A., REID, J. J., BECHDEL, S. I., BORLAND, A. A., BEAM, A. L., AND WILLIAMS, P. S. Prepartum Milking. *J. Dairy Sci.*, **27**: 638-639. 1944.
- (3) ROWLAND, S. J. The Determination of the Nitrogen Distribution in Milk. *J. Dairy Research*, **9**: 42-46. 1938.
- (4) SMITH, V. R., AND BLOSSER, T. H. Parturient Paresis. I. The Incidence of Parturient Paresis and Changes in the Total Blood Serum Calcium at Parturition in Prepartum-milked Cows. *J. Dairy Sci.*, **30**: 861-866. 1947.
- (5) TURNER, C. W. The Development of the Mammary Gland as Indicated by the Initiation and Increase in the Yield of Secretion. *Missouri Agr. Expt. Sta. Research Bull.* **156**. 1931.
- (6) TURNER, C. W. The Physiology of Milk Secretion. In *Bovine Mastitis*. Edited by Little and Plastringe, McGraw-Hill Book Company, Inc., New York, N. Y. 1946.

EFFECT OF RAW SOYBEANS AND SOYBEAN OIL MEAL ON THE VITAMIN A AND CAROTENE CONCENTRATIONS IN THE BLOOD PLASMA AND MILK OF LACTATING COWS¹

R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN

Iowa State College, Ames

In a previous trial (6) it was noted that the carotene concentration in the blood plasma of lactating cows was reduced when the cows were fed 9 lb. of ground, raw soybeans daily. When only the oil portion of the beans was used, the reduction in the carotene concentration in the blood plasma was somewhat less than when the whole bean was fed. Because of this difference, it was reasoned that soybean oil meal probably carried some factor or factors affecting the blood plasma carotene.

This trial was designed to learn whether soybean oil meal when fed to dairy cows would cause their blood plasma carotene to fall as was the case when soybeans or soybean oil was fed. Also, the experiment was to recheck the effects of cracked raw soybeans on the carotene in the blood plasma of lactating cows.

EXPERIMENTAL PROCEDURE

Nine Holstein cows which had been on pasture were assigned to three similar experimental groups. The trial was initiated with a preliminary period of 2 weeks in which each animal was fed daily 40 lb. of corn silage, and from 16 to 18 lb. of concentrate. The amount of concentrate, based on milk yield, was established at the beginning and remained the same throughout the trial. The concentrate was made up of two parts: (a) 3 lb. of old process linseed oil meal and (b) enough of a basal mixture consisting of 400 lb. ground yellow corn, 400 lb. crushed oats, 200 lb. wheat bran, 12 lb. salt and 12 lb. bone meal to bring the total up to the requirements for the cow.

The preliminary period was followed by an experimental period of 9 weeks. The same ration used in the preliminary period was fed to one group of cows; the second group received a similar concentrate except that 7.2 lb. of expeller process oil meal replaced 4.2 lb. of the basal grain and 3 lb. of linseed oil meal. The third group of cows had 6 lb. of the basal mixture and 3 lb. of linseed oil meal replaced by 9 lb. of raw soybeans, which were ground fresh every 10 days. When the oil is extracted from 9 lb. of soybeans, there are 7.2 lb. of oil meal left. This accounts for the different amounts of soybean products used.

Blood and milk samples were collected weekly at the same hour from each of the experimental cows. The blood samples were analyzed for vitamin A and carotene² according to a method previously described (6). The vitamin A and

Received for publication February 4, 1949.

¹ Journal paper no. J964 of the Iowa Agricultural Experiment Station, Ames. Project no. 692.

² A Klett-Summerson photoelectric colorimeter was employed for all the analyses. The instrument was equipped with a narrow band 440 m μ filter for estimating the carotenoids and 550 m μ narrow band filter for estimating the vitamin A.

carotene contents of the milk were determined by a modification of the Boyer *et al.* (2) method. The modification consisted of using activated glycerol dichlorohydrin (G.D.H.) (5) instead of antimony trichloride as the colorimetric reagent for estimating the vitamin A content of the milk. Duplicate fat tests were made on each of the milk samples using the standard Babcock method. The total fat of each milk sample was calculated according to the method of Berl and Peterson (1).

The values for carotene and vitamin A that were found in the blood plasma and milk the second week of the basal period were used as base points from which all changes were measured after the cows were placed on the experimental rations.

The 9-week experimental period was followed by an additional 3 weeks in which all experimental conditions were maintained except that each cow was fed 100,000 units of vitamin A³ daily by capsule.

RESULTS AND DISCUSSION

The average concentrations of carotene and vitamin A in the blood plasma and milk fat of all groups of cows are presented in table 1. Carotene values of both the blood plasma and milk fat for all groups of cows declined during the first 4 to 6 weeks of the trial. The plasma carotene concentrations of the control group and the group fed soybean oil meal then tended to level off while that of the cows fed raw soybeans continued to drop before becoming relatively constant.

The rapid lowering of the blood plasma carotene concentrations observed in all groups during the first 4 weeks of the trial is to be expected in dairy cows taken off pasture and limited to silage and concentrate. In a previous trial (6) the effect of feeding raw soybeans on the concentration of blood plasma carotene was apparent after feeding the beans 1 week. In this trial the effect of the raw soybeans was not apparent until the fifth week of the experiment. It was not until after the initial rate of decline in the blood plasma carotene of all groups that the "depressing" effect of the raw soybeans became apparent.

The vitamin A of the blood plasma and of the milk fat did not fluctuate nearly so much as the carotene. The vitamin A concentration trends were similar for all rations.

As can be noted from table 1, the feeding of raw soybeans caused the blood plasma carotene concentrations to be depressed to a greater extent than was the case when the cows were fed either the control ration which contained no soybean products or the ration containing soybean oil meal. This depression is similar to that noted in another experiment (6) but the differences are not so large. This variation between the results of the two trials might have been due to the supplemental carotene fed in the first trial.

The differences in the concentrations of carotene in the blood plasma and milk fat obtained from the cows fed the control ration and the cows fed soybean oil meal were small and probably unimportant. In a previous trial (6), the feeding of soybean oil had an effect on the blood plasma carotene intermediate

³ Fish oil containing 25,000 U.S.P. units of vitamin A per g., obtained from White Laboratory, Newark, N.J.

TABLE 1
Average vitamin A and carotene concentration in the blood plasma and milk fat of cows fed the control, soybean oil meal and raw soybean rations

Wk.	Control			Soybean oil meal			Raw soybeans		
	Milk fat		Plasma	Milk fat		Plasma	Milk fat		Plasma
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene
	($\gamma/g.$)		($\gamma/100$ ml.)	($\gamma/g.$)		($\gamma/100$ ml.)	($\gamma/g.$)		($\gamma/100$ ml.)
	Basal Period (2 wk.)								
1	4.26	6.46	391	5.92	7.24	492	5.06	7.52	434
2	3.93	5.16	326	5.21	6.62	397	4.45	6.82	351
	Experimental period (9 wk.)								
3	3.15	6.04	262	4.21	7.06	298	2.73	5.87	264
4	2.21	5.96	177	3.62	7.20	262	1.76	6.50	204
5	2.18	4.70	138	3.43	7.11	239	2.64	6.19	143
6	2.12	4.90	131	3.29	7.00	258	2.66	6.27	128
7	1.92	6.35	149	3.40	6.62	259	1.81	5.76	113
8	2.79	5.37	165	3.50	7.58	292	1.26	5.65	120
9	2.36	6.43	208	4.04	7.75	325	1.45	5.95	153
10	2.40	7.01	249	3.47	7.11	347	1.59	6.27	141
11	2.91	6.25	240	3.09	6.85	294	1.47	5.04	124
	Vitamin A Supplemental period (3 wk.)								
12	2.52	7.75	229	2.85	8.13	250	0.98	7.44	122
13	2.87	7.09	226	3.45	7.01	237	1.64	7.02	125
14	2.85	8.87	238	2.85	8.62	265	1.65	7.41	125

between that of the raw soybeans and the control ration. Because of this intermediate effect it was supposed that the soybean oil meal would carry portions of the factor or factors in raw soybeans which cause a depression of blood plasma carotene concentrations.

Since the same basal ration was fed with differences only in the protein supplement, it was assumed that all of the cows consumed similar quantities of carotene and vitamin A. The data show that the ration effect was displayed in carotene differences rather than in vitamin A differences. The cows that were fed the control and the soybean oil meal rations showed larger concentrations of carotene in their blood plasma and also excreted more carotene in their milk than the cows fed the soybean ration. Presumably raw soybeans either were causing destruction of carotene in the digestive tract or were interfering with its absorption into the blood stream.

It seems logical to assume that there was little vitamin A *per se* in the rations so that the cows on all rations were maintaining their vitamin A plasma and milk concentrations by converting carotene after it was absorbed into the body. This left unanswered the question as to whether there were differences among the rations in their effects on vitamin A absorption.

The supplementation of the ration of each cow in all groups with 100,000 U.S.P. units of vitamin A during the last 3 weeks of this trial caused the vitamin A content of the blood and milk fat of all cows to rise. Since the rate and direction of change in vitamin A content was approximately the same with all cows, with differences statistically insignificant, it was assumed that these rations did not affect vitamin A metabolism as they did carotene.

The data furnish no conclusive evidence that soybean products may not affect vitamin A absorption. Shaw *et al.* (3) found symptoms of a vitamin A deficiency when cows were fed a ration that was low in carotene and contained raw soybean meal. Possibly the daily intake of 100,000 U.S.P. units of Vitamin A was sufficient to cover any depressing effects of the soybean products on vitamin A, or the quantity of carotenoids present in the gastro-intestinal area of the cow might have exercised a sparing effect. Sherman (4) has shown that xanthophyll fed to rats may effect a sparing action on vitamin A *per se*.

SUMMARY

A reduction of the carotene concentration of the blood plasma and of the milk of lactating cows resulted when raw soybeans were fed.

When soybean oil meal was fed in an amount equivalent to that of the raw beans minus their oil content, the carotene concentrations in the blood plasma and the milk fat were similar to those when the cows were fed the control ration.

Both the control and soybean oil meal groups excreted more carotene in their milk than did the group fed raw soybeans.

Loss of these nutrients from the bodies of the cows fed raw soybeans thus was not the cause of the observed reduction of their blood plasma carotene concentration. Apparently soybeans either interfered with carotene absorption or caused some destruction of it.

No significant differences were observed among the groups of cows in the quantity of vitamin A *per se* in the blood plasma and in the milk during the last 3 weeks of the trial when all the rations were supplemented with 100,000 U.S.P. units of vitamin A daily.

REFERENCES

- (1) BERL, S., AND PETERSON, W. H. Distribution of Carotene and Vitamin A in Butter-making. *J. Dairy Sci.*, **28**: 103-107. 1945.
- (2) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk—A Rapid Extraction Procedure. *Ind. Eng. Chem., Anal. Ed.*, **16**: 101-102. 1944.
- (3) SHAW, J. C., MOORE, L. A., AND SYKES, J. F. Vitamin A Deficiency in Dairy Cattle on Rations Containing Ground Raw Soybeans. *J. Dairy Sci.*, **30**: 566-569. 1947.
- (4) SHEERMAN, W. C. Relative Gastro-intestinal Stability of Carotene and Vitamin A and Protective Effect of Xanthophyll. *Proc. Soc. Exptl. Biol. Med.*, **65**: 207-210. 1947.
- (5) SOBEL, A. E., AND WERBIN, H. Activated Glycerol Dichlorohydrin—A New Colorimetric Reagent for Vitamin A. *Ind. Eng. Chem., Anal. Ed.*, **18**: 570. 1946.
- (6) SQUIBB, R. L., CANNON, C. Y., AND ALLEN, R. S. Effect of Raw Soybeans and of Soybean Oil on Plasma Carotene and on Vitamin A as Measured by Activated Glycerol Dichlorohydrin. *J. Dairy Sci.*, **31**: 421-427. 1948.

THE ADAPTATION OF A STANDARD CURVE TO THE TURBIDOMETRIC METHOD OF ASSAY OF HYALURONIDASE IN BULL SEMEN¹

JOHN P. MIXNER AND JAMES E. JOHNSTON
New Jersey Agricultural Experiment Station, Sussex

The turbidometric method for the assay of the enzyme hyaluronidase has been modified for the assay of semen by Leonard *et al.* (3). The turbidity reducing unit (TRU) has been used in this assay as the measure of hyaluronidase activity. It generally is recognized that the TRU is not an absolute unit of hyaluronidase activity but varies according to the substrate and blood serum proteins used, the pH control, salt concentration, etc. Dorfman and Ott (1) and Warren *et al.* (4) have developed modifications of the turbidometric method which tend to make the TRU more reproducible.

The turbidometric method of assaying bull semen hyaluronidase in terms of weight equivalent of a partly purified preparation of hyaluronidase and a standard curve may have several advantages over the expression of activity in terms of the TRU. In addition to greater facility in calculating activity of hyaluronidase preparations by use of the standard curve, use of a purified preparation of hyaluronidase lends itself to comparative studies among laboratories by the simple expediency of exchanging hyaluronidase preparations. This is in lieu of an international unit of hyaluronidase.

The purpose of this report is to show that bull semen hyaluronidase assays obtained by using several dilutions of seminal plasma correspond closely to a standard curve derived from use of a purified hyaluronidase preparation obtained from bull testes when assayed turbidometrically.

METHODS

Semen for this study was obtained from dairy bulls in a stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex, N. J. Partly purified bull testes hyaluronidase (30 TRU per mg.) was used for the hyaluronidase enzyme standard², and a single preparation of highly purified potassium hyaluronate² was used as the enzyme substrate.

Blood serum was prepared from the blood of a single cow to have a uniform supply. Relatively large quantities of blood were collected at a time and the serum was bottled in 15 ml. serum bottles and kept frozen until needed. No trouble has been experienced, however, in shifting from one lot of blood serum to another, if the serum was aged properly before and after its dilution.

The turbidometric assay for the hyaluronidase was conducted essentially as

Received for publication February 8, 1949.

¹ Paper of the journal series, New Jersey Agricultural Experiment Station, Rutgers University-The State University of New Jersey, Department of Dairy Industry.

² The potassium hyaluronate and the bull testes hyaluronidase were furnished through the courtesy of Dr. D. Roy McCullagh, Schering Corporation, Bloomfield, N. J.

described by Leonard *et al.* (3) for bull semen and powdered hyaluronidase. Only 5 minutes, however, were allowed for the development of turbidity after the addition of acidified blood serum. A Klett-Summerson photoelectric colorimeter with red filter no. 66 was used for measuring turbidities. Enzyme incubation was carried out in a water bath regulated to 37° C.

RESULTS AND DISCUSSION

Standard bull testes hyaluronidase curve. To obtain a regression of hyaluronidase activity on turbidity readings, ten enzyme dosages (0.05 mg. to 0.5 mg.) were incubated with 0.2 mg. potassium hyaluronate and subsequently acidified blood serum was added to cause turbidity development. Each enzyme dosage determination was made three times (two exceptions) for a total of 28 determinations. The correlation between hyaluronidase dosage in mg. and turbidity readings was -0.985 ± 0.006 . A standard curve (fig. 1) was constructed from

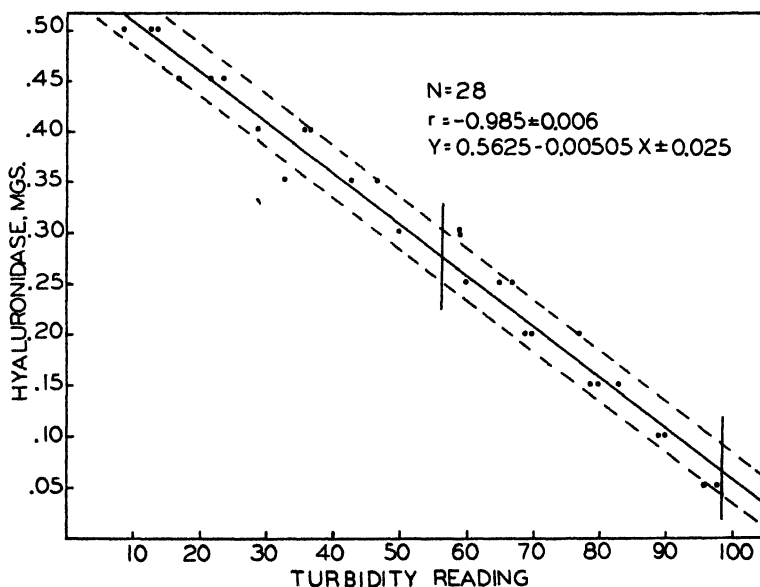


FIG. 1. Regression line obtained by correlating colorimeter turbidity readings with mg. of hyaluronidase. Solid line is the regression line, parallel broken lines are the standard errors of estimate. Vertical solid lines are the turbidity levels of the 0.2 mg. and 0.1 mg. hyaluronate standards.

use of a regression equation obtained by the method of least squares.

Individual data points are shown as well as the standard error of estimate of the regression equation. Solid vertical lines crossing the regression line indicate the turbidity readings of 99 for the 0.2 mg. hyaluronate standard and 57 for the 0.1 mg. hyaluronate standard. According to this, 1 TRU is equivalent to 0.275 mg. of hyaluronidase. The regression equation, Y (hyaluronidase, mg.) = $0.5625 -$

0.00505 X (turbidity reading), was used for converting semen assay turbidity data to mg. equivalent of standard hyaluronidase.

Standard hyaluronidase curve and semen hyaluronidase activity. Semen samples were assayed initially within 1 hour after ejaculation and again after 24 hours of incubation at 37° C. (Johnston and Mixner, 2). Certain standard semen dilution rates were used during assay on each semen sample. In the initial assays, dilution rates 1: 39, 56 and 100 as well as 1: 56, 72 and 100 were used. In like manner, dilution rates 1: 160, 200 and 267 and 1: 200, 267 and 400 were used for incubated semen. As an example of calculation of semen hyaluronidase potency per ml., if a 1: 100 dilution of a semen sample gives a turbidity value of 50, then the mg. equivalent of hyaluronidase per ml. of original semen would be 100×0.31 mg. (mg. equivalent for turbidity value of 50) = 31 mg. If three different dilution rates are used with a given semen sample, the final mg. equivalent of hyaluronidase should be the same for all three dilution rates.

A total of 76 semen samples was assayed for hyaluronidase equivalent (table

TABLE 1

Correspondence of semen hyaluronidase assays to standard hyaluronidase assay regression line

No. of semen samples	23			7			17			29		
Type of assay	Initial			Initial			Incubated 24 hr. 37° C.			Incubated 24 hr. 37° C.		
Assay dilution rates 1:	39	56	100	56	72	100	160	200	267	200	267	400
Hyaluronidase activity (mg. per ml.)	33.1	33.1	30.6	37.3	35.1	37.0	103.0	98.8	99.8	130.5	127.2	139.8

1) using three dilution rates for each sample. Average hyaluronidase activity in terms of mg. per ml. are shown for the samples at the various dilution rates. Analysis of variance revealed that there were no significant differences in potencies exhibited within the four comparisons made at their respective dilution rates.

Turbidity reducing units and mg. of hyaluronidase. One hundred seventeen semen samples were assayed for hyaluronidase, and hyaluronidase activities were calculated both in terms of TRU's and as mg. of standard hyaluronidase equivalent. The coefficient of correlation between these two measures of activity was $+0.954 \pm 0.008$, showing their essential sameness. The derived regression equation indicated that 1 TRU = 0.274 mg. of hyaluronidase, which is nearly identical to the TRU value obtained from data presented in figure 1.

The validity of expressing hyaluronidase potencies in semen in terms of a standard hyaluronidase preparation is shown by the results reported.

SUMMARY

In using the turbidometric assay for hyaluronidase, considerable flexibility in the calculation of hyaluronidase potency in semen is achieved by reference to a standard preparation of hyaluronidase. The coefficient of correlation between

mg. of standard hyaluronidase and colorimeter meter reading was -0.985 ± 0.006 .

Hyaluronidase assay values of semen in terms of mg. of standard hyaluronidase, obtained by diluting semen at various rates, conformed to the standard regression line of purified hyaluronidase.

A coefficient of correlation of $+0.954 \pm 0.008$ was obtained between turbidity reducing units and mg. of standard hyaluronidase equivalent for 117 semen samples, indicating the essential sameness of the two measures of hyaluronidase potency.

The results indicate the validity of expressing semen hyaluronidase potencies in terms of milligrams of a standard hyaluronidase preparation.

REFERENCES

- (1) DORFMAN, A., AND OTT, M. K. Turbidometric Method for the Assay of Hyaluronidase. *J. Biol. Chem.*, **172**: 367-375. 1948.
- (2) JOHNSTON, J. E., AND MIXNER, J. P. Development of Hyaluronidase in Bull Semen. *J. Animal Sci.*, **7**: 440-446. 1948.
- (3) LEONARD, S. L., PERLMAN, P. L., AND KURZBOK, R. A Turbidometric Method for Determining Hyaluronidase in Semen and Tissue Extracts. *Endocrinology*, **39**: 261-269. 1946.
- (4) WARREN, G. H., DURSO, J. G., AND LEVIN, N. R. A Modified Turbidometric Method for the Assay of Hyaluronidase. *Endocrinology*, **43**: 48-51. 1948.

HYALURONIDASE RELATIONSHIPS IN DAIRY BULL SEMEN¹

JAMES E. JOHNSTON, EDWARD J. STONE AND JOHN P. MIXNER

New Jersey Agricultural Experiment Station, Sussex

The possibility of a relationship between the hyaluronidase content and fertilizing capacity of semen has led us to investigate the correlations existing between hyaluronidase levels and other characteristics of dairy bull semen and also to determine whether significant differences in hyaluronidase levels exist among different breeds of bulls and among individual bulls.

Werthessen *et al.* (13) found no change in hyaluronidase concentration in human semen which had been stored for as long as 2 weeks unless putrefaction had occurred. Hechter and Hadidian (1) have shown that rabbit spermatozoa are capable of liberating hyaluronidase, and Johnston and Mixner (3) have reported increases as great as 400 per cent in hyaluronidase concentration in bull semen upon storage at either 5 or 37° C.

Kurzrok *et al.* (4), Werthessen *et al.* (13) and Swyer (12) have reported a direct relationship between sperm concentration and hyaluronidase concentration in human semen, and Sallman and Birkeland (11) have reported a correlation coefficient of +0.28 between these factors in dairy bull semen. The latter assays for hyaluronidase were conducted within 20 hours after collection of the semen.

METHODS

Semen samples were collected with an artificial vagina from a group of Holstein, Guernsey and Brown Swiss bulls in the stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex. One hundred semen ejaculates were used in obtaining all data except those involving percentages of live sperm, in which case only 50 ejaculates were used.

Seminal hyaluronidase was assayed by the turbidometric method as outlined by Leonard *et al.* (6) except for the use of a standard curve in obtaining hyaluronidase activity (Mixner and Johnston, 8). In this modification, semen hyaluronidase potencies are referred to a standard curve obtained by using a preparation of bull testes hyaluronidase² (30 TRU per mg.) and using potassium hyaluronate² as the substrate. Semen hyaluronidase concentrations therefore are reported as mg. equivalent of this preparation of hyaluronidase. Turbidities were measured with a Klett-Summerson photoelectric colorimeter with red filter no. 66.

Semen samples were assayed for hyaluronidase initially within 1 hour after ejaculation and again after storage for 24 hours at 37° C. A drop of toluene was added to each sample stored at 37° C. to inhibit bacterial growth. These time

Received for publication February 8, 1949.

¹ Paper of the journal series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey—, Department of Dairy Industry.

² The bull testes hyaluronidase and potassium hyaluronate were furnished through the courtesy of Dr. D. Roy McCullagh, Schering Corporation, Bloomfield, N. J.

intervals were adhered to rigidly, since Johnston and Mixner (3) have shown that the hyaluronidase level in bull semen changes significantly on storage at either 5 or 37° C.

Undiluted semen. Semen was collected by the use of the artificial vagina in a graduated test tube and the volume read at the time of collection. A photo-electric turbidometric procedure similar to that proposed by Salisbury *et al.* (10) was used in determining sperm concentration. Semen was rated for motility on a 5 to 0 scale (5 = highest motility, 0 = no motility) as proposed by Herman and Swanson (2), using the hanging drop technic and employing a 38° C. microscope stage incubator. Motility initially was rated within 1 hour after ejaculation. Semen then was cooled slowly to 5° C. and rated daily until motility ceased.

Diluted semen. A portion of each semen sample was diluted 1:10 with an egg yolk-citrate dilutor containing 3 mg. sulfanilamide per ml. Diluted samples were

TABLE 1
Characterization of semen samples

Semen character	Mean and s.e.	Standard deviation	Range
Undiluted semen			
Initial hyaluronidase (mg./ml.)	40.3 ± 1.5	15.4	9-77
24-hr. hyaluronidase (mg./ml.)	124.4 ± 4.4	44.3	35-290
Sperm concentration (millions/ml.)	1382.8 ± 45.3	452.6	262-2309
Semen volume (ml.)	5.2 ± 0.2	2.0	0.8-12.0
Sperm/ejaculate (billions)	7.3 ± 0.4	4.0	0.3-23.2
Initial motility	3.4 ± 0.1	1.0	1-5
Duration of "2" motility (hr.)	138.8 ± 5.9	58.8	0-276
Total duration of motility (hr.)	228.2 ± 10.4	103.8	36-612
Diluted semen			
Total duration of motility (hr.)	420.0 ± 13.9	139.4	108-876
Initial live sperm (%)	64.5 ± 1.8	12.5	29-87
Live sperm surviving cold shock (%)	48.8 ± 2.3	16.2	10-83

cooled slowly to 5° C. and motility examinations made as for undiluted samples. The initial percentage of live sperm was determined on freshly diluted semen with a Fast Green FCF-Eosin Y stain as proposed by Mayer *et al.* (7). For the determination of the percentage of live sperm following a cold shock, 0.1 ml. of freshly diluted semen was placed in 0° C. ice water bath for 10 minutes and then stained immediately as before.

RESULTS AND DISCUSSION

The mean values, standard deviations and ranges for each of the semen characters studied are presented in table 1.

The hyaluronidase data were grouped by breeds of bulls and by individual bulls (for summary see table 2). An analysis of variance of the original data indicated that there were no significant breed differences in mg. of hyaluronidase per ml. of seminal plasma assayed initially and also after 24 hours, but that there were highly significant differences in these characters among individual bulls.

When adjustment was made for differences in sperm concentrations of the various ejaculates, using the analysis of covariance technic, highly significant

TABLE 2

Mean hyaluronidase levels in semen initially and at 24 hr., arranged by breeds and bulls

Breed	Bull no.	No. semen samples	Hyaluronidase content of semen	
			Initial assay ^a	24-hr. assay ^a
			(mg./ml.)	(mg./ml.)
Guernsey	1	8	51.1	155.5
	2	7	51.0	129.9
	3	6	23.7	103.8
	4	8	34.1	112.8
	5	4	54.8	174.5
	Mean	—	42.9	135.3
Brown Swiss	6	8	50.9	153.3
	7	6	33.5	103.2
	Mean	—	42.2	128.3
Holstein	8	7	22.0	92.6
	9	9	52.7	180.3
	10	4	51.5	141.3
	11	9	35.0	102.6
	12	5	20.8	67.4
	13	6	45.0	123.2
	14	6	37.3	134.7
	Mean	—	37.8	120.3

^a Differences among bulls significant at the 1% level.

differences still were shown among the initial hyaluronidase levels of bulls but not among the 24-hour hyaluronidase levels. This indicates that significant differences do not exist among the average total amounts of hyaluronidase per

TABLE 3

Correlation coefficients obtained between hyaluronidase levels and other semen characters

Semen character	Zero order correlations with:		First order partial correlations independent of sperm concentration with:	
	Initial assay	24-hr. assay	Initial assay	24-hr. assay
Undiluted semen				
Initial hyaluronidase	..	+ 0.64**	..	+ 0.48**
Sperm concentration	+ 0.54**	+ 0.70**		
Sperm/ejaculate	+ 0.48**	+ 0.54**	+ 0.18	+ 0.18
Semen volume	+ 0.16	+ 0.21*	+ 0.07	+ 0.09
Initial motility	+ 0.04	+ 0.25*	- 0.07	+ 0.11
Duration "2" motility	+ 0.01	+ 0.002	+ 0.03	- 0.02
Total duration motility	+ 0.09	- 0.14	+ 0.01	- 0.03
Diluted semen				
Total duration motility	+ 0.09	+ 0.30**	- 0.06	- 0.12
Initial live sperm (%)	- 0.19	+ 0.16	- 0.30*	- 0.08
Live sperm surviving cold shock (%)	- 0.37**	- 0.16	- 0.38**	- 0.13

* Significant at 5% level.

** Significant at 1% level.

individual sperm among the bulls studied as measured by the 24-hour 37° C. incubation assay.

To determine the relationships existing between hyaluronidase levels (initial and 24-hour assays) and other semen characters, zero order coefficients of correlation were calculated on the original ungrouped data (table 3). Since sperm concentrations showed highly significant correlations of +0.54 with initial hyaluronidase levels and +0.70 with 24-hour hyaluronidase levels, first order partial correlations independent of sperm concentration also were derived.

The highly significant coefficients of correlation obtained between sperm concentration and hyaluronidase levels support the observations of Kurzrok *et al.* (4), Werthessen *et al.* (13) and Swyer (12) to this effect. The coefficients of correlation obtained are considerably higher than that obtained (+0.28) by Sallman and Birkeland (11) in dairy bulls. The differences may be explained by

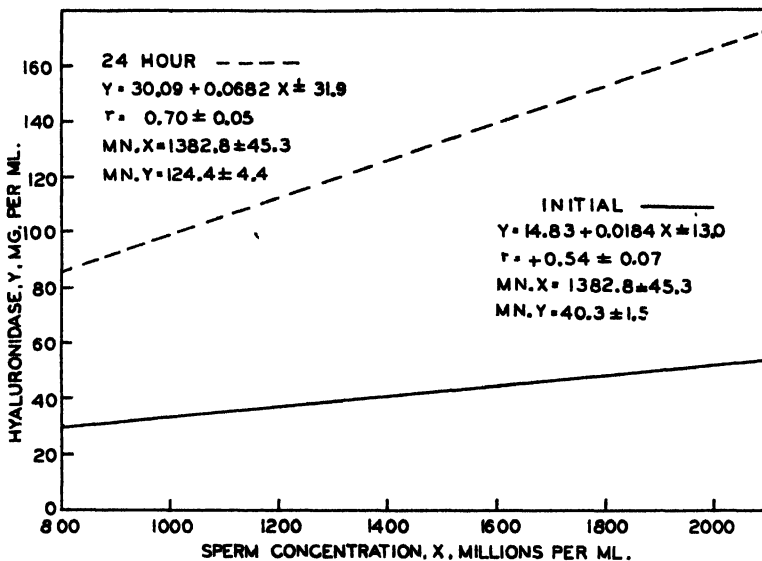


Fig. 1. Regression of hyaluronidase levels both initially and at 24 hours on sperm concentration.

the fact that the assays included in the present analysis were run at fixed intervals after ejaculation while Sallman and Birkeland assayed within a 20-hour period after ejaculation, disregarding the variable effect of semen storage on hyaluronidase levels (Johnston and Mixner, 3).

The regressions of hyaluronidase levels both initially and at 24 hours on sperm concentration are shown graphically in figure 1.

Of the first order partial correlations (independent of the effect of sperm concentration only the correlations of initial hyaluronidase with initial percentage live sperm and with percentage live sperm surviving cold shock have significance. These significant correlations may be explained when the scheme of release of

hyaluronidase from spermatozoa is considered. Johnston and Mixner (3) and Perlman *et al.* (9) have observed that as motility of a semen sample decreases and the sperm die, the hyaluronidase level increases. Since initial percentage live sperm and the percentage of live sperm surviving a cold shock are related inversely to the number of weak, dead and dying spermatozoa, one would expect significant negative correlations between these measures and the initial hyaluronidase levels of semen when they are independent of the effect of sperm concentration.

SUMMARY

One hundred semen samples from 22 dairy bulls (including Guernsey, Brown Swiss and Holstein) were assayed for hyaluronidase within 1 hour after ejaculation and again after incubation for 24 hours at 37° C. Analysis of variance indicated that, although there were no significant differences among the breeds in hyaluronidase levels (either initial or after 24 hours), there were highly significant differences among individual bulls. However, when adjustment was made for the effect of sperm concentration through the analysis of covariance, there were no significant differences among bulls in respect to the 24-hour hyaluronidase levels, whereas the initial hyaluronidase levels still showed highly significant differences.

Correlation coefficients between seminal hyaluronidase levels (both initial and 24-hour assays) and the following semen characteristics were determined: *undiluted semen*: sperm concentration, sperm per ejaculate, semen volume, initial motility, duration of "2" motility and total duration of motility; *diluted semen*: total duration of motility, initial percentage live sperm and percentage live sperm surviving cold shock. Although many of these zero order correlations were significant or highly significant, when first order partial correlations (independent of sperm concentration) were used, only the negative partial correlations of initial hyaluronidase with initial percentage live sperm and with percentage live sperm surviving cold shock retained significance.

REFERENCES

- (1) HECHTER, O., AND HADIDIAN, Z. Hyaluronidase Activity of Spermatozoa. *Endocrinology*, 41: 204-205. 1947.
- (2) HERMAN, H. A., AND SWANSON, E. W. Variations in Dairy Bull Semen with Respect to its Use in Artificial Insemination. *Mo. Agr. Expt. Sta. Research Bull.* 326. 1941.
- (3) JOHNSTON, J. E., AND MIXNER, J. P. The Development of Hyaluronidase in Bull Semen. *J. Animal Sci.*, 7: 440-446. 1948.
- (4) KURZROK, R., LEONARD, S. L., AND CONRAD, H. Role of Hyaluronidase in Human Infertility. *Am. J. Med.*, 1: 491-506. 1946.
- (5) LASLEY, J. F., AND BOGART, R. Some Factors Influencing Reproductive Efficiency of Range Cattle Under Artificial and Natural Breeding Conditions. *Mo. Agr. Expt. Sta. Research Bull.* 376. 1943.
- (6) LEONARD, S. L., PERLMAN, P. L., AND KURZROK, R. A Turbidometric Method for Determining Hyaluronidase in Semen and Tissue Extracts. *Endocrinology*, 39: 261-269. 1946.
- (7) MAYER, D. T., SQUIERS, D., AND BOGART, R. An Investigation of the Staining Principle and the Background Stain in the Differentiation of Live from Dead Spermatozoa. (Abstract) *J. Animal Sci.*, 6: 499. 1947.

- (8) MIXNER, J. P., AND JOHNSTON, J. E. The Adaptation of a Standard Curve to the Turbidometric Method of Assay of Hyaluronidase in Bull Semen. *J. Dairy Sci.*, **32**: 570-573. 1949.
- (9) PERLMAN, P. L., LEONARD, S. L., AND KURZROK, R. Some Factors Influencing the Liberation of Hyaluronidase from Testes Homogenate and Spermatozoa in the Rat. *Endocrinology*, **42**: 26-30. 1948.
- (10) SALISBURY, G. W., BECK, G. H., ELLIOTT, J., AND WILLETT, E. L. Rapid Methods for Estimating the Number of Spermatozoa in Bull Semen. *J. Dairy Sci.*, **26**: 69-78. 1943.
- (11) SALLMAN, B., AND BIRKELAND, J. M. Interrelationships of Spermatozoa Count, Hyaluronidase Titre and Fertilization. *Am. J. Physiol.*, **152**: 271-279. 1948.
- (12) SWYER, G. I. M. The Release of Hyaluronidase from Spermatozoa. *Biochem. J.*, **41**: 413-417. 1947.
- (13) WERTHESEN, N. T., BERMAN, S., GREENBERG, B. E., AND GARGILL, S. J. A Technique for the Assay Hyaluronidase in Human Semen and Its Correlation with the Sperm Concentration. *J. Urol.*, **54**: 565-570. 1945.

A PROPOSED METHOD FOR THE DETERMINATION OF COLOR OF DRY PRODUCTS OF MILK¹

R. P. CHOI, A. F. KONCUS, C. M. O'MALLEY AND B. W. FAIRBANKS

The American Dry Milk Institute, Inc., Chicago

One of the important characteristics by which the consumer judges the acceptability of dry products of milk is the color of the products. Factors such as excessive exposure to heat during processing, high moisture content and prolonged storage time at high temperature are generally known to promote browning. A rapid and reproducible method for estimating the extent of discoloration is desirable from the standpoint of quality control.

In their study of the factors influencing the development of color in evaporated milk, Webb and Holm (5) employed the Munsell color system of visual examination, using permanent color standards prepared from ferric chloride and potassium dichromate. A procedure for extracting color from browned nonfat dry milk solids and dry whey solids using a concentrated solution of trisodium phosphate and sodium chloride was reported by Doob *et al.* (1). Experience with this method indicated that only a small fraction of the color is extracted from browned nonfat dry milk solids. A method involving the measurement of reflectance by means of the Beckman Spectrophotometer recently was reported by Nelson (3) for the determination of color of evaporated milk and related products.

One of the factors influencing the extraction of color from dry products of milk is the rather strong adsorption of the color by casein. Kass and Palmer (2) showed that the adsorption follows Freundlich's equation, $\frac{x}{m} = KC^{\frac{1}{n}}$ where x is the amount of material adsorbed, m is the weight of the adsorbent, C is the equilibrium concentration of the adsorbate and K and n are constants. Since the amount adsorbed depends directly on the concentration of the adsorbent, it is reasonable to believe that by breaking down some of the large casein molecules extraction of the color may be more complete. Hydrolysis of the protein molecules may be accomplished easily at ordinary temperatures by means of proteolytic enzymes with minimum danger of further color production during the hydrolysis. Consequently, when the remaining proteins, proteoses and peptones are precipitated, the filtrate should contain most, if not all, of the undesirable color in addition to the small amount of water-soluble, natural chromogenic materials of milk. The following procedure was developed based upon this principle.

Received for publication February 16, 1949.

¹ The subject matter of this paper has been undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors and do not necessarily reflect the views or endorsement of the Department of the Army.

PROCEDURE

Ten g. of the dry product of milk were dispersed in distilled water and made up to a total volume of 100 ml. A 25-ml. portion was transferred to a 125-ml. Erlenmeyer flask containing 1.5 ml. of a 10 per cent trypsin² suspension. The sample was incubated for 1 hour at 45° C., after which 1 ml. of 50 per cent trichloroacetic acid and approximately 0.1 g. of Celite Analytical filter-aid (Johns-Manville) were added to the digested mixture. The mixture was filtered and transmission determined on the clear filtrate by means of a Pfaltz and Bauer

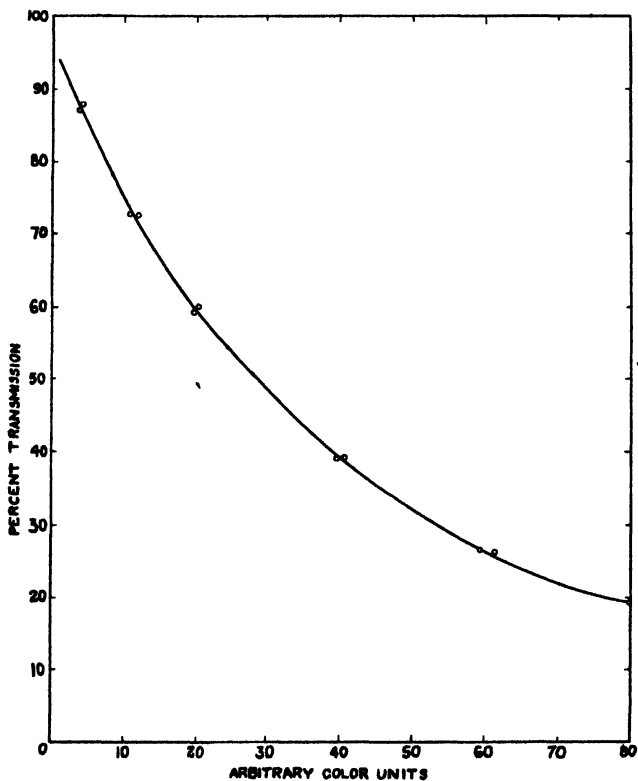


FIG. 1. Standard curve constructed with iodine in 10% potassium iodide solution. 1 mg. iodine per 50 ml. equals 1 color unit.

fluorophotometer with filter no. 485. Correction was made for the small amount of color due to the enzyme by setting the enzyme blank at 100 per cent transmission.

To permit comparison of results among different laboratories it was necessary to standardize the transmission readings against an arbitrary but reproducible standard. After consideration of different colored compounds, it was decided that a solution of iodine in 10 per cent potassium iodide would be the most satis-

² Wilson Laboratories, Chicago, Illinois.

factory standard from the standpoints of reproducibility, similarity of color and ease of preparation, the only disadvantage being that the color of this standard does not follow Beer's law in the wave-length region chosen. The standard curve was determined by dissolving 200 mg. of resublimed iodine (Baker's C. P.) in a small volume of freshly prepared 10 per cent potassium iodide solution and diluting to 100 ml. in a volumetric flask with the same potassium iodide solution. Different volumes of this solution were transferred to 50-ml. volumetric flasks and made up to volume with 10 per cent potassium iodide solution. After thorough mixing the transmission readings were determined with a potassium iodide blank set at 100 per cent transmission. The color of a solution containing 1 mg. of iodine per 50 ml. was arbitrarily designated as one color unit. On this basis the standard curve in figure 1 was constructed. Since 25 ml. of the reconstituted sample contained 2.5 g. of the dry product, the number of color units per gram was calculated by dividing the color units obtained from the standard curve by 2.5.

RESULTS AND DISCUSSION

The effect of enzyme concentration was investigated using a sample of nonfat dry milk solids of high moisture content which had been browned on laboratory storage at 37° C. The enzyme concentration was varied from 0 to 400 mg. per 25 ml. of reliquefied sample, and the incubation period was fixed at 1 hour. The result of each determination was corrected for the color due to the enzyme. To estimate the extent of hydrolysis at each enzyme concentration, non-protein nitrogen was determined by the method of Rowland (4).

From the results presented in table 1 it is evident that hydrolysis of the pro-

TABLE 1

The effect of trypsin concentration on the extent of color liberation and protein hydrolysis

Trypsin concentration	Color	Non-protein N
(mg./25 milk)	(extinction)	(% of dry milk)
0	0.135	0.274
25	0.260	1.79
50	0.335	2.41
100	0.378	2.95
150	0.407	3.63
200	0.392	3.74
400	0.385	3.72

tein greatly facilitates the liberation of color. With the incubation time fixed at 1 hour, increasing the enzyme concentration increases both the color extinction and the non-protein nitrogen. Maximum color liberation appears to have been reached at a trypsin concentration of 150 mg. per 25 ml. of milk. Further increases in enzyme concentration do not seem to have appreciable effects on either color extinction or non-protein nitrogen content.

The rate of color liberation was determined using a concentration of trypsin of 150 mg. per 25 ml. of a reconstituted sample of badly-browned nonfat dry milk solids. The amount of color extracted was determined at varying incubation

periods up to 5 hours with correction made for the slight decrease in volume resulting from evaporation. Results are plotted in figure 2, along with the corresponding non-protein nitrogen results.

With the enzyme concentration kept constant at 150 mg. per 25 ml. of milk, the color liberated and the non-protein nitrogen value both increase with the time of incubation, rapidly in the first hour and slower thereafter until a maximum and constant value appears to be reached after 3 hours. It is difficult to decide whether the color extracted at this point represents the total color or merely a portion of the total color which is extractable under these conditions. The fact that the precipitate from trichloroacetic acid is colored only slightly indicates that

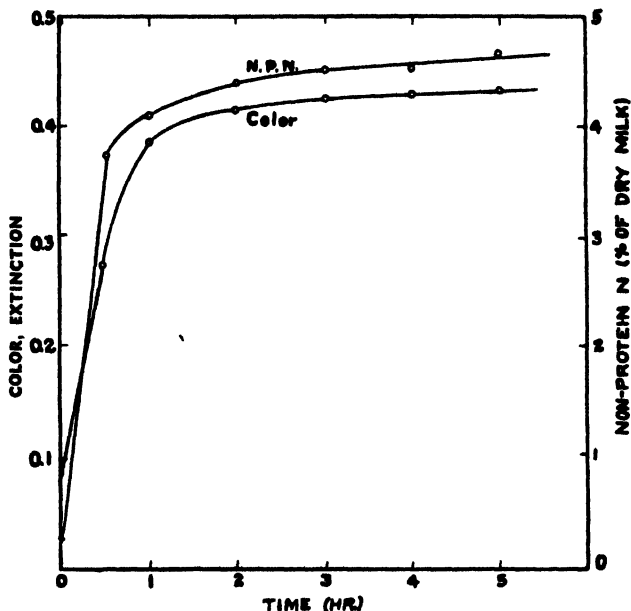


FIG. 2. Rates of color liberation and protein hydrolysis using 150 mg. trypsin per 25 ml. of reconstituted nonfat dry milk solids.

most, if not all, of the color has been extracted. As a 3-hour incubation period would be inconveniently long, a 1-hour period was adopted in the procedure with slight sacrifice in the completeness of color extraction. However, it must be pointed out that the sample used in this experiment is colored much more highly than the worst ones normally encountered, so that the difference in color between 3- and 1-hour incubation ordinarily would be inappreciable. The parallelism which exists between the amount of color extracted and the non-protein nitrogen developed emphasizes the importance of color adsorption by the protein molecules.

As Celite analytical filter-aid was used in the procedure to increase filtration rate and to prevent occasional turbidity, there is a possibility that this material may adsorb a certain part of the liberated color. If appreciable adsorption occurs, the color in the filtrate should decrease with increasing amounts of the filter-

aid used. No appreciable effect on the amount of color extracted was detected when as high as 0.3 g. of filter-aid per sample was used. The use of this material, while not necessary in the cases of nonfat dry milk solids and dry whole milk, is important in some dry whey solids and dry buttermilk solids in order to eliminate turbidity in the filtrate.

TABLE 2
Comparison of the enzyme procedure with the alkaline extraction procedure

Sample		Color extinction using:	
Type	No.	Alkaline extraction	Enzyme
Nonfat dry milk solids	1	0.055	0.125
	2	0.148	0.138
	3	0.060	0.136
	4	0.031	0.150
Dry whey solids	1	0.096	0.081
	2	0.031	0.185
	3	0.172	0.185
	4	0.090	0.130

A comparison of the enzyme procedure with the alkaline extraction procedure of Doob *et al.* (1) for several samples of dry whey solids and browned nonfat dry milk solids was made. The weight of the sample and the volume of liquid used in the enzyme procedure were adjusted to those employed in the alkaline extraction

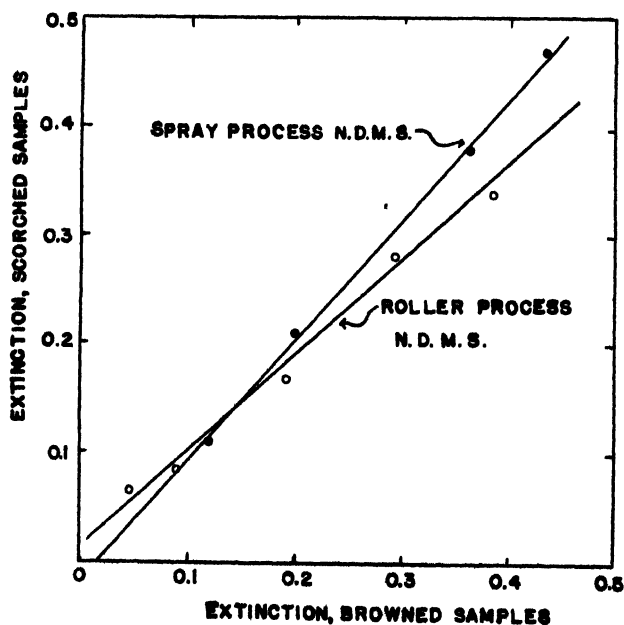


FIG. 3. Relationship between the extractable color of samples containing varying amounts of nonfat dry milk solids scorched at 119° C. and extractable color of visually matched samples similarly prepared from nonfat dry milk solids browned at 37° C. under the influence of high moisture content.

procedure. Data are presented in table 2. It should be noted that, while agreement between the two methods is fair in the case of dry whey solids, the enzyme method is distinctly more efficient in the extraction of color from nonfat dry milk solids. Since dry whey solids normally contain little or no casein, these results again point to the strong influence of casein on color extraction.

The brown discoloration in dry products of milk may arise from storage at ordinary temperature and high moisture content or from scorching in the drying operation. Results of the above experiments show that the present method is applicable to samples browned under storage. To determine whether the method can be applied equally well to products scorched during processing the following series of experiments was performed. A sample of spray process and a sample of roller process nonfat dry milk solids were used. A portion of each was allowed to attain a high moisture content in a humid atmosphere and then browned by storing at 37° C. Another portion of each was scorched in an

TABLE 3
Color of some samples of dry products of milk

Sample		Extinction	Color units (/g. solids)
Type	No.		
Dry whole milk	1	0.057	0.88
	2	0.054	0.76
	3	0.047	0.60
	4	0.042	0.48
Spray nonfat dry milk solids	1	0.068	1.28
	2	0.100	2.32
	3	0.110	2.56
	4	0.095	2.16
Roller nonfat dry milk solids	1	0.130	3.36
	2	0.100	2.32
	3	0.115	2.76
	4	0.130	3.36
Dry butter-milk solids	1	0.222	7.28
	2	0.390	14.28
	3	0.096	2.20
	4	0.142	4.68

air oven at 119° C. for 3 hours. For the scorched sample of each product a series of mixtures of varying color intensity and known composition were prepared by mixing different amounts of the scorched material with the original product. Another series of mixtures was prepared similarly from the browned product to match the color of each of the scorched mixtures. Matching was done visually. In figure 3 the extractable color of the scorched samples expressed as extinction is plotted against that of the corresponding matched browned sample. With both types of nonfat dry milk solids a straight line of approximately unit slope is obtained. This indicates that the present method works equally well for scorched products and for products browned at ordinary temperatures with high moisture contents. It may be mentioned that the color extinction was found to

be a linear function of the concentration of either the browned or the scorched nonfat dry milk solids in the mixture.

In table 3 the arbitrary color units of some random samples of dry products of milk are presented. Differences between the various products are in part due to differences in the concentration of the natural color materials.

The method is simple and precise and may be applicable to other dairy products.

SUMMARY

Since the chief difficulty associated with the extraction of color from browned dry products of milk is the strong adsorption of the color by the proteins, it has been found that most, if not all, of the color could be liberated by extensive hydrolysis of the milk proteins with trypsin. A simple and reproducible method has been developed which involves measuring photometrically the color of a trichloroacetic acid filtrate of the hydrolyzed mixture and expressing the color intensity in terms of an arbitrary color standard of iodine in potassium iodide solution. The method works equally well for samples experimentally browned under storage with high moisture contents and for samples experimentally scorched at high temperature.

REFERENCES

- (1) DOOB, H., JR., WILLMANN, A., AND SHARP, P. F. Influence of Moisture on Browning of Dried Whey and Skim Milk. *Ind. Eng. Chem.*, **34**: 1460-1468. 1942.
- (2) KASS, J. P., AND PALMER, L. S. Browning of Autoclaved Milk. *Ind. Eng. Chem.*, **32**: 1360-1366. 1940.
- (3) NELSON, V. The Spectrophotometric Determination of the Color of Milk. *J. Dairy Sci.*, **31**: 409-414. 1948.
- (4) ROWLAND, S. J. The Determination of Nitrogen Distribution in Milk. *J. Dairy Research*, **9**: 42-46. 1938.
- (5) WEBB, B. H., and HOLM, G. E. Color of Evaporated Milks. *J. Dairy Sci.*, **13**: 25-39. 1930.

THE CAROTENE AND VITAMIN A AND PROXIMATE COMPOSITION OF PORTIONS OF THE FIRST MILKING POSTPARTUM¹

H. D. EATON, R. E. JOHNSON, L. D. MATTERSON AND A. A. SPIELMAN

Animal Industries Department, Storrs Agricultural Experiment Station, Storrs, Connecticut

Under different systems of management, calves receive, immediately after birth, different portions of colostrum which may differ markedly in nutritive value. This was indicated directly by data obtained by Spielman *et al.* (16) on the first milking postpartum from three cows; successive increments increased markedly in the content of fat, carotene and vitamin A. Previously, several workers (7, 14, 18) had noted that in a small percentage of cows, the first milking postpartum contains less carotene and vitamin A than succeeding milkings. They have attributed this to incomplete "let-down" of milk at the first milking. During the lactation period, in general, the first milk drawn contains, under most conditions, appreciably less fat than that obtained at the end of the milking (1, 6), but the other nutrients are believed to remain constant during the milking process. Successive portions of human milk obtained during a single nursing period (10, 19) show a similar increase in fat and also a change in other constituents.

The objectives of this study were to determine the trends which occur in the carotene and vitamin A content and in the proximate constituents of successive 2-lb. increments of the colostrum from the first milking postpartum of dairy cows. Secondly, these trends have been related to two different experimental systems of management and feeding.

EXPERIMENTAL

Animals. This experiment was conducted on 24 cows of the Ayrshire, Guernsey, Holstein and Jersey breeds in the University of Connecticut herd which calved from January to June, 1948. They represented four experimental groupings which were equalized so far as possible in respect to breed, age, number of previous lactations, length of dry period, health, ancestry and previous dietary history. Group 1-A received the basal ration and was milked postpartum, group 1-B received the basal ration plus 1 million U.S.P. units of vitamin A daily for 30 days prepartum and was milked postpartum, group 2-A received the basal ration and was milked prepartum and group 2-B received the basal ration plus 1 million U.S.P. units of vitamin A daily for 30 days prepartum and was milked prepartum.

For 8 weeks prior to the calculated parturition date, all cows received the same basal ration fed on the basis of liveweight. One lb. of U. S. no. 2 alfalfa

Received for publication February 19, 1949.

¹ This work was supported in part by the Big-Y-Foundation, Norwich, Conn., and Chas. M. Cox Co., Boston, Mass.

hay, 3 lb. of well-matured corn silage and 1 lb. of a grain mixture consisting largely of cereal grains and containing approximately 13.5 per cent crude protein was fed per 100 lb. of liveweight. The hay, silage and grain contained on an average 3.87 mg., 1.06 mg. and 0.15 mg. of carotene per pound, respectively, as determined by the method of Moore and Ely (11) as modified by Nelson *et al.* (13). The vitamin A supplement was shark liver oil² containing 25 per cent by weight of crude soybean lecithin. This oil contained 54,440 U.S.P. units of vitamin A per g. as assayed spectrophotometrically against the U.S.P. vitamin A reference standard (vitamin A acetate in cottonseed oil). The cows in the prepartum-milked groups were milked twice daily, starting 10 days prior to the calculated date of parturition. They actually were milked for an average of 8.8 days with a standard error of ± 1.1 days. The newborn calves were not allowed to nurse but were removed immediately to a separate portion of the barn.

Samples. Most cows were milked immediately after calving and in all cases within 4 hours. The first eight cows were milked by hand and the others by machine, but all cows were hand stripped. Samples were taken of each successive 2 lb. of colostrum. All except the last sample represented a minimum of 2 lb. of colostrum, while the maximum amount did not exceed 2.2 lb. Colostrum samples were held at 4° C. pending analysis which, in most cases, was completed within 4 days after collection.

Analyses. The carotene and vitamin A content of the colostrum were determined by the method of Boyer *et al.* (4) using an Evelyn photo-electric macrocolorimeter. The colorimeter was standardized with crystalline carotene in petroleum ether (b. p. 30–60° C.) and with crystalline vitamin A alcohol in chloroform. Specific gravity, protein, fat and ash were determined by the methods of the Association of Official Agricultural Chemists (2). Lactose was determined by the micromethod of Hawk *et al.* (8) with the following modifications. To obtain the protein-free filtrate, 2 ml. each of 5 per cent ZnSO_4 and 0.3 *N* $\text{Ba}(\text{OH})_2$ as used in Somogyi's blood sugar method (15), were added to 0.5 ml. of colostrum. One ml. of this filtrate plus 2 ml. of Somogyi's copper reagent then were placed in boiling water for 10 minutes. After cooling, 1 ml. of Nelson's arsenous molybdate reagent (12) was added and the volume made to 25 ml. with H_2O . The resulting solution then was read in the Evelyn photo-electric macrocolorimeter against a standard lactose solution which had been treated in the same manner as the protein-free filtrate. All determinations were made and are expressed on a volumetric basis.

The composition of a given nutrient in the successive 2 lb. increments usually followed a linear trend, either positive or negative. Therefore, the magnitude of this trend was determined by least squares to determine the characteristic slope for each nutrient in consecutive samples from each cow. An example of the calculation follows:

² This oil was supplied by Mr. Melvin Hochberg of the Nopec Chemical Company, Harrison, New Jersey.

Increment	Amount of colostrum (lb.)	Midpoint of milking interval in % (X)	$\bar{X} - \bar{X}$ (x)	% Protein (y)	(xy)
1st	2	12.99	-38.5	20.35	-783.475
2nd	2	38.97	-12.5	19.08	-238.500
3rd	2	64.95	13.5	18.31	247.185
4th	1.7	88.98	37.5	17.48	655.500
Total (S)	7.7	205.89	0	-119.290
Mean (\bar{X})		51.47			

$$\text{Slope} = b = \frac{S(xy)}{S(x^2)} = \frac{-119.290}{3227.00} = -0.036966$$

$$\text{Effect of Slope } (B^2) = \frac{S^2(xy)}{S(x^2)} = 4.4907 \text{ (in dimensions of } y^2)$$

Calculation of each slope (b) and its effect (B^2) upon the variation of y was determined as above. An analysis of variance was used to test the significance of the combined slope for all treatments and of differences in slope between treatments. Since standard methods of statistical analysis (2) were used, the detailed analysis of variance will not be given.

TABLE 1

Data on trend in constituents of colostrum during first milking postpartum for individual cows

Group	Cow no.	Total colostrum	Denominator for slope $S(x^2)$	Trend on successive increments (X) of milk or slope (b)						
				Specific gravity	Protein	Lactose	Fat	Ash	Carotene	Vitamin A
		(lb.)			(g. %)	(g. %)	(g. %)	(g. %)	(γ%)	(γ%)
1A	1a	20.5	7961.56	-0.1282	-0.022	-0.004	+0.11	-0.0012	+4.1	+4.6
	2	14.0	5708.58	-0.1351	-0.017	-0.005	+0.10	-0.0019	+6.5	+4.0
	3	7.7	3227.00	-0.2177	-0.037	+0.001	+0.06	-0.0012	+1.4	+4.8
	4	16.0	6582.52	-0.1056	+0.004	-0.011	+0.11	-0.0014	+1.1	+3.1
	5	16.0	6582.52	-0.1636	-0.002	-0.012	+0.15	-0.0026	+2.4	+9.1
	6	16.0	6582.52	-0.0837	-0.015	-0.005	+0.09	-0.0010	+6.3	+1.1
	7	16.0	6582.52	-0.1322	-0.009	-0.007	+0.16	-0.0014	+2.2	+6.0
1B	8	11.0	5388.16	-0.0502	+0.010	-0.007	+0.03	-0.0001	+1.1	+4.8
	9	27.5	11982.16	-0.1429	-0.022	-0.006	+0.11	-0.0018	+3.7	+24.4
	10a	22.0	9065.48	-0.1192	-0.017	-0.004	+0.11	-0.0011	+1.3	+10.0
	11	6.0	2217.78	+0.0601	-0.003	+0.003	+0.05	-0.0004	+1.0	+7.0
	12	10.0	4000.00	+0.0600	-0.004	+0.002	+0.03	+0.0005	+0.4	+3.3
	13	20.0	8250.00	-0.1436	-0.028	-0.006	+0.16	-0.0017	+2.9	+10.5
2A	14	18.0	7423.72	-0.1423	-0.005	-0.008	+0.09	-0.0011	+0.3	+0.8
	15	14.0	5708.58	-0.0825	+0.001	-0.003	+0.06	+0.0002	+1.0	+1.2
	16	10.5	3816.42	-0.0613	-0.002	-0.010	+0.05	-0.0007	+3.0	+1.5
	17a	15.4	6898.92	-0.1127	-0.004	-0.006	+0.11	-0.0009	+0.7	+1.1
	18	7.2	2046.98	-0.0339	-0.004	+0.004	+0.04	-0.0003	+1.2	+0.6
2B	19a	20.0	8250.00	-0.1000	-0.002	-0.008	+0.08	-0.0004	+0.6	+3.2
	20	13.2	4387.96	-0.0812	-0.000	-0.007	+0.12	-0.0002	+0.6	+4.2
	21	20.0	8250.00	-0.0588	+0.016	-0.008	+0.12	+0.0007	+2.8	+10.7
	22	9.8	4080.80	-0.0391	-0.007	0.000	+0.05	-0.0004	+1.6	+8.4
	23	13.0	4449.04	-0.0222	+0.003	+0.004	+0.04	+0.0006	+0.4	+2.3
	24	9.4	4259.94	-0.0127	-0.007	+0.004	+0.05	+0.0001	+0.3	+1.8

* Data of these cows plotted in figures 1 to 4.

RESULTS

Data on the trend in the proximate constituents and in carotene and vitamin A for each individual cow are given in table 1. The results from a representative cow from each of the four experimental groups are plotted in figures 1

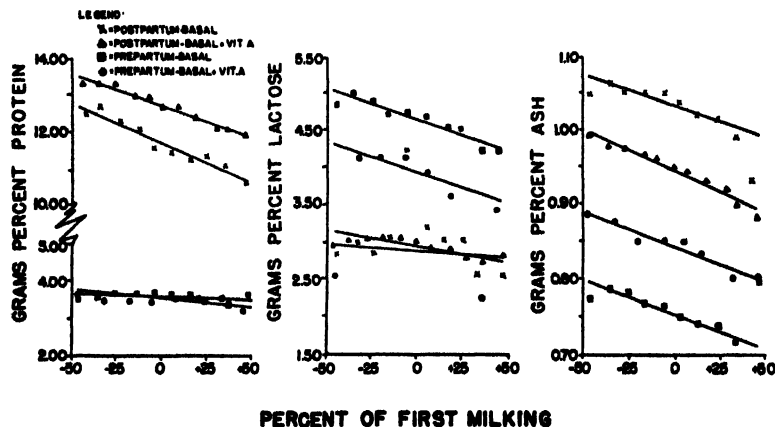


FIG. 1. The changes in the per cent protein, lactose and ash of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

through 4 against per cent of total colostrum for each constituent which was determined. With the exception of γ vitamin A per g. of fat, all constituents showed significant trends ($P < 0.01$) with successive 2-lb. increments of colostrum drawn.

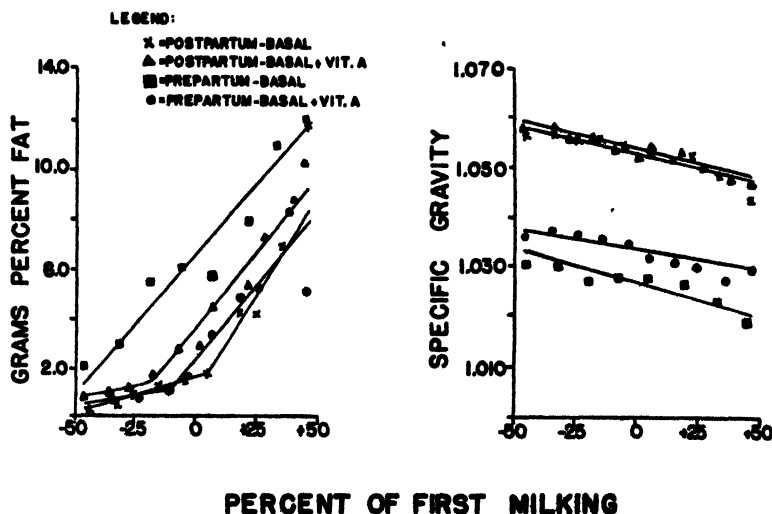


FIG. 2. The changes in the per cent fat and specific gravity of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

The protein, lactose and ash (fig. 1) showed a highly significant negative trend ($P < 0.005$). For those cows milked only postpartum, the protein and ash decreased more rapidly ($P < 0.01$) than in cows milked prepartum. The addition of vitamin A had no significant effect on the trends. In terms of absolute amounts the trends in successive increments of colostrum are relatively small.

The fat content rose markedly (fig. 2) with successive increments of colostrum, while specific gravity decreased. Both trends were highly significant ($P < 0.001$). While the decrease in specific gravity was greater ($P < 0.05$) in those cows milked only postpartum as compared to those milked prepartum, the addition of vitamin A had no significant effect. In contrast to protein, lactose and ash, the quantitative changes in the fat content were relatively large.

Both the per cent carotene and vitamin A content (fig. 3) increased markedly

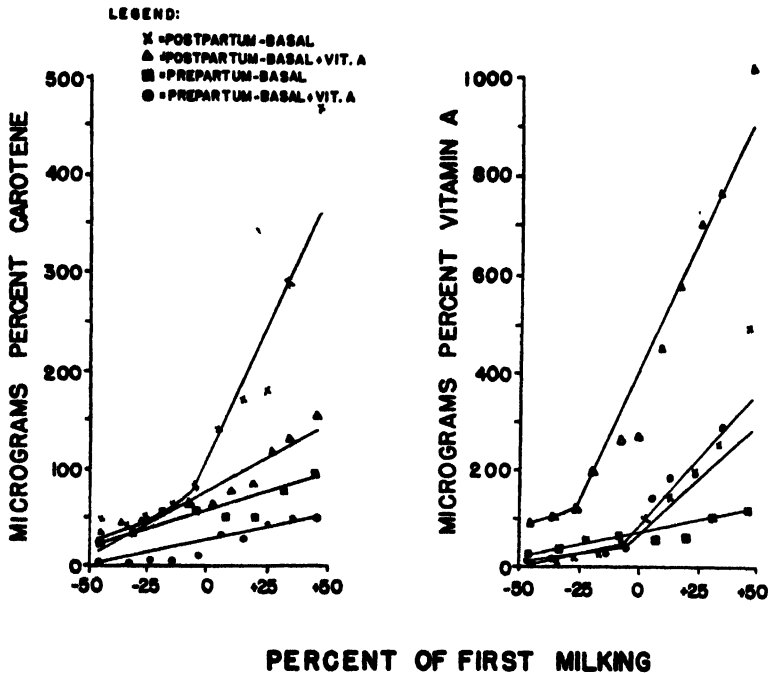


FIG. 3. The changes in the per cent carotene and vitamin A of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

with successive increments of colostrum with highly significant trends ($P < 0.001$). Both carotene ($P < 0.025$) and vitamin A ($P < 0.05$) increased at a greater rate with successive increments of colostrum in the cows milked only postpartum than in those cows milked prepartum. The addition of supplementary vitamin A had no significant effect on the trend in per cent carotene. However, the vitamin A content in the colostrum from the cows receiving supple-

mentary vitamin A increased more rapidly ($P < 0.05$ for postpartum group and $P < 0.025$ for prepartum group) than in that from the cows receiving the basal ration alone. Quantitatively, the changes in both carotene and vitamin A were relatively large.

When the concentration of vitamin A was computed per gram of fat (fig. 4),

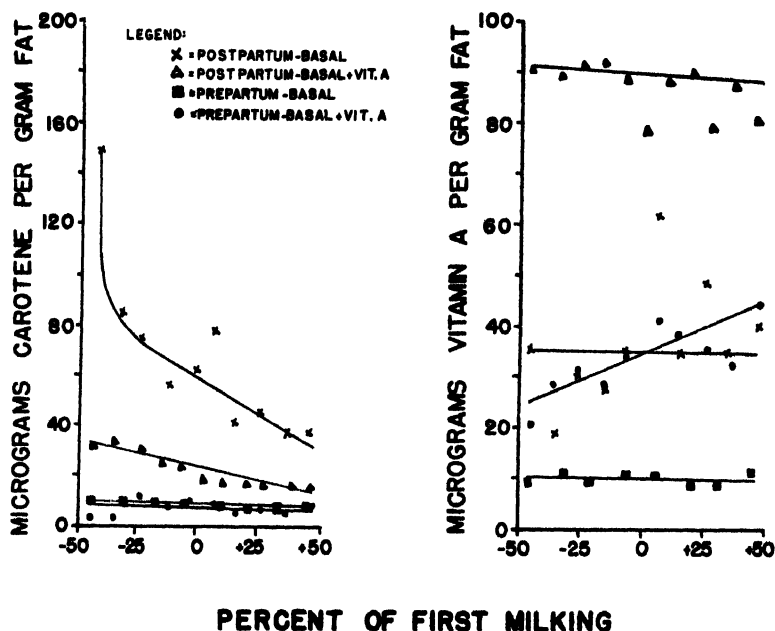


FIG. 4. The changes in the carotene and vitamin A content per g. of fat of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

there were no significant trends with successive increments of colostrum, whereas the concentration of carotene per gram of fat showed a decreasing trend ($P < 0.01$) with successive increments. This trend was not affected significantly by either pre- or postpartum milking or by the addition of vitamin A to the basal ration.

DISCUSSION

These data indicate that definite trends do occur in the carotene and vitamin A content and in the proximate constituents in consecutive increments of the colostrum from the first milking postpartum. In cases of udder congestion at parturition, where complete milking may be indicated, milking the cow first and then allowing the calf to nurse would seem to insure optimum vitamin A intake to the calf.

The decrease in the per cent protein, lactose and ash of successive increments of the first milking postpartum is relatively small quantitatively. However, the variation in the various nitrogenous fractions making up the protein contents, especially the globulin fraction, would be of interest. The same also is true of individual minerals making up the per cent ash. Studies (10, 19) on samples of human milk representing various portions of a single nursing period have shown that protein tends to increase slightly, that intermediate portions tend to be higher in lactose than initial or final portions, and that ash tends to follow the same trends in the intermediate portions but is slightly lower at the end than at the beginning.

The increase in the per cent fat with successive increments of colostrum from the first milking postpartum is in agreement with the excellent summary by Espe (6) of similar studies later in lactation. A similar rise in the carotene and vitamin A content has been suggested by other workers (7, 14, 18) although no actual data were given.

The decreasing trend in the carotene content per gram of fat and the relative constancy of the vitamin A content per gram of fat are of interest. The decrease in both the carotene and vitamin A in the blood plasma at the time of parturition, as reported by Sutton *et al.* (17) and others, and the increase in the per cent carotene content of colostrum occurring in cows milked prepartum (17, 5) are suggestive of more active mammary metabolism of these substances. Possibly the levels of plasma carotene and vitamin A at the time of calving are independent of mammary influence but are affected by other factors possibly hormonal (20) in nature.

SUMMARY

The carotene and vitamin A content and the proximate constituents of successive 2-lb. increments of the first milking postpartum have been studied on 24 cows. These represented two managerial groups of which one was milked for an average of 8.8 days prepartum, while the other was milked only postpartum. Some of the cows in each of the above groups received a basal ration only and the others received the same basal ration plus 1 million U.S.P. units of vitamin A daily for 30 days prepartum. The following trends were observed:

1. Per cent protein, lactose and ash and specific gravity showed a significant negative trend with successive increments of colostrum. The per cent of protein and of ash and specific gravity decreased at a significantly greater rate in the cows milked postpartum than in those milked prepartum. Quantitatively the changes were relatively minor.

2. Per cent fat, carotene and vitamin A showed a significant positive trend with successive increments of colostrum. In the cows milked postpartum, the increase in per cent carotene and vitamin A was significantly greater than that found in cows milked prepartum. The addition of supplementary vitamin A had no effect on the trends for carotene but caused a more rapid rate of in-

crease in the percentage content of vitamin A than that observed in cows receiving the basal ration alone.

3. The carotene content per gram of fat showed a significant negative trend with successive increments of colostrum, whereas no significant trends were observed in the content of vitamin A per gram of fat.

ACKNOWLEDGEMENTS

The authors are most grateful to Messrs. F. Warren and G. Farrington for the care of the experimental animals and to Misses M. W. Dicks and J. H. Kramer and Messrs. R. J. Slate, J. Satchell and L. Nezvesky for technical assistance at various times during the course of the experiment.

Further acknowledgment is due Dr. C. I. Bliss, Storrs Agricultural Experiment Station Biometrician, for considerable aid in the statistical analysis of the data and in the preparation of the paper, and to Mrs. L. Griswold, Department of Poultry Husbandry, for the carotene analysis of the feedstuffs.

REFERENCES

- (1) ASSOCIATES OF L. A. ROGERS. *Fundamentals of Dairy Science*. 2nd ed. Reinhold Publishing Company, New York, N. Y. 1935.
- (2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. *Official and Tentative Methods of Analysis*. 6th ed. Washington, D. C. 1945.
- (3) BLISS, C. I., AND ALLMARK, M. G. The Digitalis Cat Assay in Relation to Rate of Injection. *J. Exptl. Ther.*, **81**: 378-389. 1944.
- (4) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. *Ind. Eng. Chem., Anal. Ed.*, **10**: 101-102. 1944.
- (5) EATON, H. D., JOHNSON, R. E., SPIELMAN, A. A., AND MATTERSON, L. D. Unpublished Data. Animal Industries Department, Storrs Agricultural Experiment Station. 1948.
- (6) ESPE, D. *Secretion of Milk*. 3rd ed. Iowa State College Press, Ames. 1946.
- (7) HANSEN, R. G., PHILLIPS, P. H., AND SMITH, V. R. Colostrum Milk and Its Vitamin A Content. *J. Dairy Sci.*, **29**: 809-814. 1946.
- (8) HAWK, P. B., OSER, B. L., AND SUMMERSON, W. H. *Practical Physiological Chemistry*. 12th ed. The Blakiston Company, Philadelphia, Pa. 1947.
- (9) KEYES, E. A., REID, J. J., BECHDEL, S. I., BORLAND, A. A., BEAM, A. L., AND WILLIAMS, P. S. Pre-Partum Milking (Abstract). *J. Dairy Sci.*, **27**: 638-639. 1944.
- (10) MACY, I. G., NIMS, B., BROWN, M., AND HUNSCHER, H. A. Human Milk Studies. VII. Chemical Analysis of Milk Representative of the Entire First and Last Halves of the Nursing Period. *Am. J. Diseases Children*, **42**: 569-589. 1931.
- (11) MOORE, L. A., AND ELY, R. Extraction of Carotene from Plant Material. A Rapid Quantitative Method. *Ind. Eng. Chem., Anal. Ed.*, **13**: 600-601. 1941.
- (12) NELSON, N. A Photometric Adaptation of the Somogyi Method for the Determination of Glucose. *J. Biol. Chem.*, **153**: 375-380. 1944.
- (13) NELSON, W. L., LOOSLI, J. K., LOFGREN, G., AND YAGER, N. Carotene Retention in Alfalfa Meal. Effect of Blanching, Packaging and Storage Temperature. *Ind. Eng. Chem.*, **40**: 2196-2198. 1948.
- (14) SARKAR, B. C. R. Carotene and Vitamin A in the Colostrum of Cows of Typical Indian Breeds. *J. Dairy Sci.*, **31**: 479-487. 1948.

- (15) SOMOGYI, M. Determination of Blood Sugar. *J. Biol. Chem.*, **160**: 69-73. 1945.
- (16) SPIELMAN, A. A., LOOSLI, J. K., AND TURK, K. L. Unpublished data. Department of Animal Husbandry, Cornell University. 1947.
- (17) SUTTON, T. S., KAESER, H. E., AND SOLDNER, P. A. Changes in the Level of Vitamin A and Carotene in the Blood Plasma of Dairy Cows Associated with Parturition and Beginning Lactation. *J. Dairy Sci.*, **28**: 933-939. 1945.
- (18) SUTTON, T. S., WARNER, R. G., AND KAESER, H. E. The Concentration and Output of Carotenoid Pigments, Vitamin A and Riboflavin in the Colostrum and Milk of Dairy Cows. *J. Dairy Sci.*, **30**: 927-932. 1947.
- (19) WAISMAN, G. S., AND PETAZZE, M. L. A. Human Milk. IV. Variations in Composition During Emptying of the Gland. *Rev. Assoc. Argentina. Dietol.*, **5**: 121-217. 1947. (*Chem. Abs.*, **42**: 7410. 1948.)
- (20) WILLIAMSON, M. B. Effect of Estrogen on Plasma Vitamin A of Normal and Thyroidectomized Rabbits. *Proc. Soc. Exptl. Biol. Med.*, **66**: 621-623. 1947.

THE COMPARATIVE VALUE OF HIGH AND LOW FAT CONCENTRATES WITH ALFALFA HAY¹

J. H. BYERS, I. R. JONES, AND J. R. HAAG²

Department of Dairy Husbandry, Oregon State College

In the alfalfa-producing areas where cows, to a great extent, are fed solely on alfalfa hay, milk production is only 50 to 85 per cent of that of cows fed alfalfa hay with grain supplements. The restricted production on alfalfa hay alone is due either to the absence of some nutritive element or elements in the alfalfa hay or to a lack of sufficient total digestible nutrients.

In an effort to determine what was responsible for this subnormal production of cows on alfalfa hay, Smith *et al.* (11) replaced a part of the alfalfa hay with equal amounts of total digestible nutrients in concentrates and noted that the greatest stimulus to milk and butterfat production was obtained when ground soybeans were fed. Since there was a greater stimulus from feeding equal total digestible nutrients in ground soybeans than from soybean meal, the question arose as to whether the stimulus resulted from protein supplementation or from the additional fat supplied.

Evidence of a conflicting nature has been presented in the literature on the effect of a ration high in dietary fat on milk and butterfat production of the dairy cow. Bratton *et al.* (1) found inclusion of raw soybeans in amounts equal to 25 per cent of the grain ration caused a measurable increase in the butterfat test of the milk produced. Williams *et al.* (13) found that cows on an all-soybean ration produced milk with a significantly higher percentage of butterfat than did cows on normal feed.

Loosli *et al.* (4), Maynard and McCay³ (5) and Maynard *et al.* (6, 7, 8) have conducted many experiments on the influence of dietary fat on milk and butterfat production. These workers concluded that the main effect of a ration high in fat is to increase the amount of milk rather than to influence the fat component of the milk. Monroe and Krauss (9), feeding grain mixtures varying from 2.69 to 4.89 per cent fat, found no differences in the production of milk, butterfat or 4 per cent fat-corrected milk. Gibson and Huffman (2) reported that when soybean oil was added to a basal ration low in fat, an increase in milk production resulted. They also reported a temporary increase in butterfat percentage.

The study reported in this paper was undertaken to investigate further the effect on milk and butterfat production of feeding a supplement high in dietary fat to cows receiving a basal ration of alfalfa hay.

Received for publication February 23, 1949.

¹ Published as technical paper no. 573 with the approval of the Director of the Oregon Agricultural Experiment Station, Corvallis.

² The authors are indebted to Dr. Jerome C. R. Li of the Mathematics Department, Oregon State College, for the statistical analysis of the data.

EXPERIMENTAL PROCEDURE

In this experiment four purebred Holstein cows were used. Two cows, 334 and 346, began the experiment on the high-fat ration and two cows, 341 and 348, on the low-fat ration. The cows were kept in stanchions throughout the experiment with the exception of about 2 hours each day when they were exercised in a cement corral. Special construction of mangers allowed for accurate measurements of feed intakes. Water was available in individual drinking bowls at all times. The cows had free access to bone meal, disodium phosphate and salt in boxes in the paved corral. The daily allowance of good quality alfalfa hay was weighed in canvas bags and fed in equal portions night and morning. Hay refused was weighed back. Ground soybeans or soybean meal was fed in equal portions twice daily. Daily feed intakes were recorded. The cows were weighed at approximately the same time each day.

The amount of alfalfa hay fed was regulated with the requirements of the animal for maintenance and production. Changes from ground soybeans to

TABLE 1
Analysis of feeds used in experiment

Feed	Ash	Mois- ture	Crude protein	Crude fiber	Ether extract	Nitrogen free extract	Diges- tible protein	Total digestible nutrients
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Alfalfa hay—1st yr. irrigated hay	7.70	7.79	14.71	30.63	2.44	36.66	10.50	51.11
Alfalfa hay—2nd yr. dry land hay	9.16	8.23	12.48	25.23	2.69	42.06	9.36	52.28
Alfalfa hay—2nd yr. irrigated hay	7.98	6.94	14.23	32.45	2.09	36.31	10.24	51.57
Soybeans	4.13	11.30	35.59	5.79	19.32	23.87	31.67	88.05
Soybean Oil Meal	5.23	13.48	40.38	5.94	4.17	30.80	34.32	76.59

soybean meal, or vice versa, were made abruptly. The amount of supplement varied with the requirements for maintenance and milk production. Total digestible nutrients consumed were greater than the requirements in every 6-week period except one.

The rations were calculated on the basis of average body weight and milk production of the 3 days prior to the beginning of each 6-week period. The mean value of the per cent fat in the dry matter in the high-fat ration was 5.2 per cent, as compared to 2.7 per cent of the low-fat ration.

The cows were milked twice daily by machine. The milk was weighed and aliquot samples were taken at each milking. The milk samples were preserved with corrosive sublimate and butterfat tests were made weekly, using the Babcock method.

The chemical analyses of the feeds used in the experiment are shown in table 1. The average digestion coefficients reported by Morrison (10) were used in calculating the total digestible nutrients and the digestible protein.

Inasmuch as the condition of the cow for a period following calving is known

to influence milk production and, more particularly, the butterfat percentage of the milk and all cows calved again within a year, the stage of lactation for which the data were used was never earlier than the 6th and never later than the 36th week of lactation.

RESULTS

The cows showed no digestive disturbances even though they were changed abruptly from one experimental ration to another. The palatability of the rations seemed to be of a high order, even though the cows ate as much as 10 lb. daily of soybeans or soybean meal. Average daily hay and concentrate consumption, total digestible nutrients required and consumed and average body weights of each individual cow are shown in table 2.

TABLE 2

Average daily hay and concentrate consumption, total digestible nutrients required and consumed and body weights for each cow by periods

Cow	Period	Hay	Concentrate	Total digestible nutrients		Body weight
				required	consumed	
		(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
334	1	40	8 SB ^a	27.5	27.5	1381
	2	40	9 SBM ^b	24.4	28.0	1391
	3	40	7 SB	22.9	27.1	1394
	4	40	6 SBM	19.9	23.1	1417
356	1	35	8 SB	25.2	25.3	1234
	2	35	8 SBM	23.5	24.7	1256
	3	35	7 SB	23.1	24.4	1270
	4	35	6 SB	19.5	23.3	1301
341	1	40	9 SBM	26.4	28.0	1341
	2	40	10 SB	25.0	29.4	1344
	3	36	8 SBM	22.4	25.1	1349
	4	36	4 SB	19.9	22.1	1375
348	1	35	8 SBM	26.2	24.4	1299
	2	35	7 SB	24.0	24.5	1268
	3	35	7 SBM	18.5	24.2	1287
	4	36	5 SB	17.2	23.2	1342

^a SB = ground soybeans.

^b SBM = soybean meal.

All four cows followed the expected curve with regard to body weight. In the early stages of their lactations each cow lost some weight, but by the second experimental period they all had begun to gain weight. As the stage of gestation advanced the experimental animals continued to gain weight normally. It may be said that the experimental ration of alfalfa hay with ground soybeans or soybean meal did not alter the expected normal gain in body weight.

The data for milk, 4 per cent fat-corrected milk, per cent butterfat and total butterfat production, as averaged by 6-week periods, are shown in table 3. For the purpose of comparing the two fat levels, the data are summarized by cows and by periods. The results are based on the fact that with three or more experimental periods of equal length, lying within a lactation where milk yield is falling at a constant rate, a comparison of the yields of a given period, with

the average of the yields of periods immediately preceding and succeeding the given period, is justified.

Figures 1 and 2 show the average daily milk production by weeks in relation to the average production of 99 lactations of Holstein cows in the Oregon State College herd (3). The average weekly butterfat test also is given for each cow.

Inasmuch as all the cows usually received more total digestible nutrients

TABLE 3

Daily average butterfat per cent, milk yield, butterfat yield and 4% fat-corrected milk yield of each cow by periods

Cow	Period	Av. daily milk yield	Av. butterfat	Av. daily butterfat yield	Av. daily 4% fat-corrected milk yield
		(lb.)	(%)	(lb.)	(lb.)
334	HF ^a I	58.7	3.35	1.955	52.9
	LF ^b II	56.5	2.76	1.567	45.4
	HF III	47.2	3.03	1.430	40.3
	LF IV	38.0	2.41	0.918	28.9
	Av. periods I and III	52.9	3.19	1.692	46.6
	Gain on HF	- 3.6	0.43	0.125	1.2
	Av. periods II and IV	47.2	2.58	1.242	37.1
	Gain on HF	0.0	0.45	0.188	3.2
356	HF I	55.2	3.48	1.920	50.9
	LF II	50.8	3.15	1.586	44.3
	HF III	44.4	3.60	1.599	41.7
	LF IV	41.3	3.20	1.326	36.4
	Av. periods I and III	49.8	3.54	1.759	46.3
	Gain on HF	- 1.0	0.39	0.173	2.0
	Av. periods II and IV	46.0	3.17	1.456	40.3
	Gain on HF	- 1.6	0.43	0.143	1.4
341	LF I	69.0	2.2	1.501	50.1
	HF II	61.2	2.6	1.591	48.3
	LF III	49.5	2.3	1.138	36.9
	HF IV	37.3	2.6	0.969	29.5
	Av. periods I and III	59.2	2.25	1.319	43.5
	Gain on HF	2.0	0.35	0.272	4.8
	Av. periods II and IV	49.2	2.6	1.270	38.9
	Gain on HF	- 0.3	0.3	0.132	2.0
348	LF I	55.4	3.6	1.969	51.7
	HF II	47.3	4.0	1.887	47.1
	LF III	43.0	2.9	1.277	36.3
	HF IV	31.0	3.0	0.940	26.5
	Av. periods I and III	49.2	3.25	1.623	44.0
	Gain on HF	- 1.9	0.75	0.264	3.1
	Av. periods II and IV	39.1	3.5	1.413	36.8
	Gain on HF	- 3.9	0.6	0.136	0.5

^a HF=high fat.

^b LF=low fat.

than they required, it may be said that any effect of the ration could not be the result of added or a lack of total digestible nutrients. In many periods the total nutrients consumed were far in excess of the required, calculated on the basis of body weight and production. The excess, however, occurred just as frequently on the low-fat as on the high-fat rations.

DISCUSSION

This study shows that good producing cows may produce at a high level on good quality alfalfa hay and supplements of the soybean plant. Production was maintained at the normal level when either ground soybeans or soybean meal was fed with alfalfa hay.

There was no significant difference in the efficiency of the ground soybeans or the soybean meal as supplements to the alfalfa hay. There are indications that the soybean meal was superior for milk production, but the differences were not significant when the Student's t-test is applied (12). Milk production on either the ground soybeans and alfalfa hay or soybean meal and alfalfa hay was significantly greater than milk production on alfalfa hay alone. The level of production was similar to the normal lactation of 99 Holstein cows in the Oregon State College herd (3).

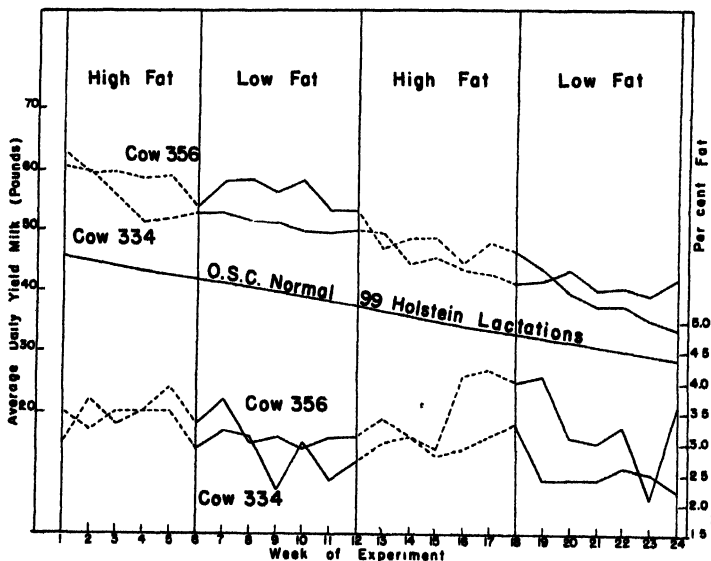


FIG. 1. Average daily milk and butterfat production by weeks for cows beginning experiment on high-fat ration.

The maximum effect of the high-fat ration was demonstrated with butterfat percentage. In every period on the ground soybeans and alfalfa hay there was an increase in the butterfat content of the milk. The percentage increase ranged from 3.4 to 27.5 per cent, which in a statistical analysis is shown to be highly significant. The Student's t-test (12) was used and all four cows showed a significant increase in per cent butterfat when fed the high-fat ration as compared to the butterfat percentage on the low-fat ration.

The influence of fat in the ration when ground soybeans were fed indicated a stimulus to 4 per cent fat-corrected milk and total butterfat production. The

results, however, could not be shown to be statistically significant. In a total of eight comparisons, soybean meal favored milk production in six. Ground soybeans favored per cent butterfat, pounds of butterfat produced and pounds of 4 per cent fat-corrected milk in all of the eight comparisons.

In this experiment considerable attention was given to the individual hay consumption of each of the cows. Loosli *et al.* (4) have presented evidence that increasing the intake of hay of each cow from a level of 1 lb. per 100 lb. of body weight to a level of 1.3 lb. of hay per 100 lb. of body weight reduced the effect of the high-fat ration from what it otherwise might have been at the lower level of hay intake. At Ohio, Monroe and Krauss (9) were unable to obtain significant differences in the comparisons they made where hay was fed *ad libitum*.

Controlled *ad libitum* feeding of U. S. No. 2 alfalfa hay was carried out in the study reported in this paper. As much as 2.5 lb. of hay per 100 lb. of body

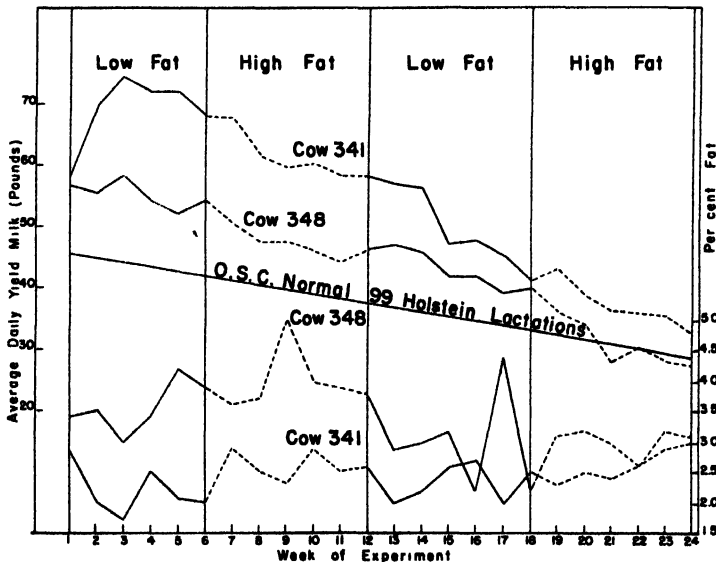


FIG. 2. Average daily milk and butterfat production by weeks for cows beginning experiment on low-fat ration.

weight was fed, although at times some hay was refused. However, the cows did eat well over 2 lb. of hay per 100 lb. of body weight throughout the greater part of the trial. The results for milk production, butterfat production and pounds of fat-corrected milk were not statistically significant for the trial as a whole, but in some instances the individual cows did show an advantage with a high-fat ration, which was significant. These results bear out the suggestion of the Cornell workers (4, 5, 6, 7, 8) that the effect of dietary fat is limited, to some extent, when the roughage consumption of the cows is at a higher level than 1 lb. of hay per 100 lb. of body weight. The results, however, do not corroborate the results of the Cornell workers who noted the greatest effect of

a high-fat ration on milk yield. Rather, this experiment indicates a slight absolute superiority of the low-fat ration for milk production. Fat-corrected milk, however, did show in every period an absolute advantage with a high-fat ration. However, the advantage was statistically significant in the case of only one cow.

In the case of the average daily production of butterfat, highly significant increases were noted in the case of one cow and significant increases in the case of a second cow. Two cows, however, did not show a significant increase, even though an absolute increase was demonstrated.

Excellent milk production for all cows was obtained in spite of a somewhat restricted ration. Some of the cows produced over 500 lb. of butterfat in 305 days and all produced well over 400 lb. It would seem, therefore, that if cows receive an ample supply of good quality alfalfa hay and a supplement containing 2 to 3 per cent dietary fat, milk production would not be significantly greater if a ration containing 4 to 5 per cent dietary fat was fed. The percentage of butterfat in the milk, however, could be expected to increase significantly with a higher per cent of fat in the ration.

SUMMARY

1. Feeding as much as 10 lb. per day of ground soybeans or soybean meal with alfalfa hay caused no digestive disturbances and the palatability of the ration was not reduced noticeably.

2. Alfalfa hay supplemented with ground soybeans or soybean meal allowed milk and butterfat production to be maintained significantly above that expected on alfalfa hay alone.

3. A ration of alfalfa hay and ground soybeans containing 5.2 per cent dietary fat did not increase milk production when compared to a ration of alfalfa hay and soybean meal containing 2.7 per cent dietary fat.

4. Significant increases in the per cent butterfat of the milk produced were obtained when the high-fat ration was fed.

5. An actual, but not significant, increase was noted in total butterfat and in pounds of 4 per cent fat-corrected milk produced.

REFERENCES

- (1) BRATTON, R. W., EPPLER, W. P., WILBUR, J. D., AND HILTON, J. H. A Study of Some of the Physico-chemical Effects of Soybeans on the Fat in Cow's Milk. *J. Dairy Sci.*, 21: 109-110. 1938.
- (2) GIBSON, G., AND HUFFMAN, C. F. The Influence of Different Levels of Fat in the Ration upon Milk and Fat Secretion. *Mich. Agr. Expt. Sta. Quarterly Bull.* 21: 4: 258. 1939.
- (3) JONES, I. R., AND CAVENDER, J. E. Normal Lactation Curve for Holstein Cows. *Oregon Agr. Expt. Sta.* 1943. Unpublished data.
- (4) LOOSLI, J. K., MAYNARD, L. A., AND LUCAS, H. L. IV. Further Studies of the Influence of Different Levels of Fat Intake upon Milk Secretion. *N. Y. (Cornell) Agr. Expt. Sta. Memoir* 265. 1944.
- (5) MAYNARD, L. A., AND MCCAY, C. M. The Influence of Different Levels of Fat Intake upon Milk Secretion. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 543. 1932.

- (6) MAYNARD, L. A., McCAY, C. M., WILLIAMS, H. H., AND MADSEN, L. L. II. Further Studies of the Influence of Different Levels of Fat Intake upon Milk Secretion. N. Y. (Cornell) Agr. Expt. Sta. Bull. 593. 1934.
- (7) MAYNARD, L. A., GARDNER, KARL E., AND HODSON, ADRIAN. Soybeans as a Source of Fat in the Dairy Ration. N. Y. (Cornell) Agr. Expt. Sta. Bull. 722. 1939.
- (8) MAYNARD, L. A., LOOSLI, J. K., AND McCAY, C. M. III. Further Studies of the Influence of Different Levels of Fat Intake upon Milk Secretion. N. Y. (Cornell) Agr. Expt. Sta. Bull. 753. 1941.
- (9) MONROE, C. F., AND KRAUSS, W. E. Relationship Between Fat Content of Dairy Grain Mixtures and Milk and Butterfat Production. Ohio Agr. Expt. Sta. Bull. 644. 1943.
- (10) MORRISON, F. B. *Feeds and Feeding*. 21st ed., unabridged. Morrison Pub. Co., Ithaca, N. Y. 1948.
- (11) SMITH, V. R., JONES, I. R., AND HAAG, J. R. Alfalfa With and Without Concentrates for Milk Production. J. Dairy Sci., 28, 5: 343-354. 1945.
- (12) SNEDECOR, GEORGE W. *Statistical Methods*. 4th ed. Iowa State College Press, Ames. 1946.
- (13) WILLIAMS, H. K., CANNON, C. Y., AND ESPE, D. L. A Soybean and Silage Ration for Dairy Cows. J. Dairy Sci., 26, 3: 241. 1943.

LIVABILITY AND FERTILITY OF BOVINE SPERMATOZOA IN DIFFERENT DILUENTS

R. W. BRATTON, R. H. FOOTE, S. D. MUSGRAVE AND N. L. VANDEMARK¹

*Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry,
Cornell University, Ithaca, N. Y.*

Considerable difference of opinion exists as to the relative merits of the available diluents for bovine semen used in artificial breeding. This is evidenced by the report of the first annual meeting of the National Association of Artificial Breeders (4) which indicates that phosphate-yolk, citrate-sulfa-yolk and Phillips' pabulum have been used with varying degrees of success.

Since the development in this country of the phosphate-yolk diluent by Phillips (5) and Phillips and Lardy (6) and the citrate-yolk formula by Salisbury *et al.* (10), the latter formula has been modified by reducing the sodium citrate dihydrate content of the buffer from 3.6 to 2.9 per cent (12) and including in it 0.6 per cent sulfanilamide (2, 9, 11). Also, Swanson (14) has reported 3 per cent sodium citrate to be satisfactory as a buffer when used with egg yolk.

A tablet form of the citrate-sulfanilamide buffer based upon Salisbury's recommendations has been prepared by the Ortho Research Foundation, Raritan, New Jersey. This same organization also has developed a ready-to-use liquid formula for bovine semen containing sodium citrate as a buffer, a sulfonamide, dextrose and other ingredients. A synthetic pabulum has been developed by Phillips and Spitzer (7) and is prepared and distributed commercially by the National Agricultural Supply Co., Fort Atkinson, Wisconsin.

With the exceptions of the early comparisons between phosphate-yolk and citrate-yolk and between citrate-yolk and citrate-sulfanilamide-yolk by Salisbury and Bratton (9), Salisbury (10) and Salisbury and Knodt (11) and the recent report of Hurst and LaMaster (1) in which phosphate-yolk, citrate-yolk and the Ortho liquid were compared, no simultaneous comparisons of the original formulae with their more recent modifications have been reported. Furthermore, the early comparisons between phosphate-yolk and citrate-yolk (10) and between phosphate-yolk and Phillips' pabulum (7) were based on extremely low dilution rates and small numbers of inseminations. The work of Salisbury (8) and Salisbury and Bratton (9) in establishing the practicability of high dilution rates with the citrate diluents has emphasized the need for re-evaluating the present day diluents at these higher rates.

In view of the lack of conclusive evidence of the relative merits of the diluents in use today in terms of spermatozoan livability and fertility at high dilution rates, the experiment reported herein was designed to compare, at dilution rates of 1:200 and using split ejaculates, the following diluents: Phillips' phosphate-yolk (6), Salisbury's citrate-yolk (10), Salisbury's citrate-sulfanilamide-yolk containing 3.6 and 2.9 per cent citrate (9, 11, 12), the Ortho Research Founda-

Received for publication March 1, 1949.

¹ Now at the University of Illinois, Urbana.

tion's buffer tablets and its ready-to-use liquid formula for bovine semen. Phillips' pabulum (7) was not used in these comparisons because unsatisfactory spermatozoan livability was encountered in preliminary storage studies in this laboratory.

EXPERIMENTAL PROCEDURE

The simultaneous comparison of all six diluents was accomplished by use of a 6×6 latin square experimental design. Each semen sample was divided into six portions and each portion diluted at a rate of 1:200 in one of the six diluents. All inseminations were made by the regularly employed technicians affiliated with the New York Artificial Breeders' Cooperative, Inc. These technicians were divided at random into six groups. Each technician group received a different diluent during 5 consecutive days of each experimental week. The remaining 2 days of each week were utilized as a change-over period. In order that all technicians would have equal opportunities for using all six diluents, and at the same time minimizing discrimination between diluents when a particular cow was to be inseminated, the experiment was conducted for a period of 6 weeks. At the dilution rates employed, the semen requirements were met by using two bulls per day, a total of ten bulls per week. Since the same ten bulls were used each week, the 6×6 latin square design was replicated ten times. The actual sequence of diluents received by technician groups each week for a single replicate is shown in table 1.

TABLE 1

Basic design of the experiment for 6 weeks using bull 1 (6×6 latin square)

Ejac.	Diluents used by technician groups					
	I	II	III	IV	V	VI
1	PY ^a	2.9 CSAY	3.6 CSAY	OTY	OL	3.6 CY
2	3.6 CSAY ^b	OL	OTY	PY	3.6 CY	2.9 CSAY
3	OL	OTY	3.6 CY	2.9 CSAY	PY	3.6 CSAY
4	3.6 CY ^b	PY	2.9 CSAY	OL	3.6 CSAY	OTY
5	OTY	3.6 CY	PY	3.6 CSAY	2.9 CSAY	OL
6	2.9 CSAY ^b	3.6 CSAY	OL	3.6 CY	OTY	PY

^a P = phosphate; C = citrate; SA = sulfanilamide; Y = egg yolk; OT = Ortho tablets; OL = Ortho liquid (used directly without egg yolk).

^b 3.6 and 2.9 indicate percentage concentration of citrate.

The semen used was obtained from Holstein bulls in the active stud of the New York Artificial Breeders' Cooperative, Inc. Semen was collected by means of the artificial vagina and examined for quality according to the routine procedures of this laboratory. Only those semen samples which contained 70 per cent or more motile spermatozoa, 900×10^6 spermatozoa per ml. of fresh semen, and reduced methylene blue in less than 9 minutes were shipped for use in insemination.

The composition of the buffers and diluents used, and the average pH of each, are shown in table 2. All diluents were prepared fresh during the afternoon preceding the morning they were to be shipped.

A 3-ml. portion of each diluted semen sample was stored at 5° C. and

examined daily for the per cent of motile spermatozoa and their relative rate of progressive movement until all the spermatozoa in the sample were dead. These examinations were used to compare the several diluents on the basis of the livability of the spermatozoa in storage.

The fertility of the diluted semen samples was determined from the 60- to 90-day non-returns to first service cows and expressed as the per cent non-

TABLE 2
Composition and pH of each buffer and diluent

	PY ^a	3.6 ^b CY	3.6 CSAY	2.9 CSAY	OTY	OL
KH ₂ PO ₄ (g.)	2.0			50	
Na ₂ HPO ₄ ·12H ₂ O (g.)	20.0				tablets	
Na ₂ C ₄ H ₇ O ₇ ·2H ₂ O (g.)		36.0	36.0	29.0		...
Sulfanilamide (g.)			6.0	6.0	
Water (redistilled over glass) to final vol. (ml.)	1000	1000	1000	1000	1000	
pH of buffer	7.36	7.41	7.36	7.41	7.20	
Ratio of egg yolk to buffer	1:1	1:1	1:1	1:1	1:1	
pH of diluent	6.76	6.70	6.72	6.73	6.67	6.52

^{a, b} See footnotes for table 1.

returns. The per cent non-returns for each ejaculate × technician group × diluent subclass (table 1) was considered as the experimental unit. These observations were subjected to the analysis of variance (3, 13) for determining statistical significance between the means for the diluents.

RESULTS

In table 3 are shown the percentages of motile spermatozoa at intervals during the first 6 days of storage at 5° C. Reliable estimates of the per cent of

TABLE 3
Livability of spermatozoa in the different diluents during storage at 5° C.

Duration of storage (hr.)	% motile spermatozoa in different diluents					
	PY ^a	3.6 ^b CY	3.6 CSAY	2.9 CSAY	OTY	OL
3	°	58	59	58	57	50
24	...	50	50	51	49	41
48	..	40	43	46	42	30
72	...	30	37	41	36	22
96	...	26	29	31	28	13
144	...	17	20	24	20	6

^{a, b} See footnotes for table 1.

° Reliable estimates not possible.

motile spermatozoa in the phosphate-yolk diluent were not possible, but in general spermatozoan livability appeared to be as satisfactory as in the other diluents. From the practical standpoint 3.6 CY, 3.6 CSAY, 2.9 CSAY and OTY were about equal for maintaining the motility of spermatozoa. The per cent

of motile spermatozoa was slightly lower in the OL diluent. However, the immotile spermatozoa were more discernible in the OL than in the yolk diluents and as a consequence the per cent of motile spermatozoa may have been estimated more reliably.

The number of first-service cows inseminated and the mean per cent 60- to 90-day non-returns for these same cows are shown in table 4. From the statistical analyses the mean percentages for non-returns for the 3.6 CSAY, 2.9 CSAY, OT and OL diluents were significantly higher (1 per cent level of probability) than those for the PY and the 3.6 CY diluents. Within the former group the differences between means were not statistically significant.

TABLE 4
Fertility level of semen in different diluents
(based on 60- to 90-day non-returns to 1st service cows)

	Diluents					
	PY ^a	3.6 ^b CY	3.6 CSAY	2.9 CSAY	OTY	OL
Total number of 1st services	1945	1847	1843	1924	1843	1810
60- to 90-day non-returns (mean %)	50.5	50.5	55.3	56.5	56.4	55.0

^{a, b} See footnotes for table, 1.

It is of particular interest that all those diluents containing sulfonamides were accompanied by non-return rates that were significantly higher than those containing no sulfonamides. The average increase in non-return rate was approximately 5 percentage units, a value similar to that reported by Salisbury and Knodt (11) when citrate-yolk was compared with citrate-sulfanilamide-yolk.

SUMMARY

By means of a 6 × 6 latin square design, six bovine semen diluents were compared. Sixty semen samples from ten Holstein bulls were subdivided into six portions and each portion diluted at a rate of 1: 200 with one of the six diluents.

Based on the per cent 60- to 90-day non-returns to approximately 1850 first service cows per diluent, the mean fertility level for each diluent was as follows: phosphate-yolk, 50.5; 3.6 citrate-yolk, 50.5; 3.6 citrate-sulfanilamide-yolk, 55.3; 2.9 citrate-sulfanilamide-yolk, 56.5; Ortho tablet-yolk, 56.4; and Ortho liquid, 55.0.

The average non-returns for the sulfonamide-containing diluents (3.6 citrate-sulfanilamide-yolk, 2.9 citrate-sulfanilamide-yolk, Ortho tablet-yolk and Ortho liquid) was 5 percentage units higher than for those diluents not containing sulfonamides (3.6 citrate-yolk and phosphate-yolk). This difference was significant at the 1 per cent level of probability.

Livability of spermatozoa during storage at 5° C. was satisfactory for all six diluents.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance and cooperation of the management and technicians of the New York Artificial Breeders' Cooperative, Inc., in furnishing the semen and in carrying out the inseminations; the cooperation of Doctor Victor R. Berliner, Director, Division of Animal Industry, Ortho Research Foundation, Raritan, N. J., in furnishing the buffer tablets and Ortho liquid diluent; and the aid of Professor C. R. Henderson in the statistical analysis of the data and in the preparation of the manuscript.

REFERENCES

- (1) HURST, V., AND LAMASTER, J. P. Results of Inseminating Dairy Cows with Semen Diluted with Phosphate, Citrate and Ortho Buffers. *J. Animal Sci.*, 7: 541. 1948.
- (2) KNOTT, C. B., AND SALISBURY, G. W. The Effect of Sulfanilamide Upon the Livability and Metabolism of Bovine Spermatozoa. *J. Dairy Sci.*, 29: 285-291. 1946.
- (3) LOVE, H. H. Experimental Methods in Agricultural Research. Published by the Agricultural Experiment Station of the University of Puerto Rico. Rio Piedras, Puerto Rico. 1943.
- (4) NATIONAL ASSOCIATION OF ARTIFICIAL BREEDERS. Report of the First Annual Meeting. Mimeographed Report. New York Artificial Breeders' Cooperative, Inc. Ithaca, New York. 1948.
- (5) PHILLIPS, P. H. Preservation of Bull Semen. *J. Biol. Chem.*, 130: 415. 1939.
- (6) PHILLIPS, P. H., AND LARDY, H. A. A Yolk-Buffer Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 23: 399-404. 1940.
- (7) PHILLIPS, P. H., AND SPITZER, R. R. A Synthetic Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 29: 407-414. 1946.
- (8) SALISBURY, G. W. Fertility of Bull Semen Diluted at 1:100. *J. Dairy Sci.*, 29: 695-697. 1946.
- (9) SALISBURY, G. W., AND BRATTON, R. W. Fertility Level of Bull Semen Diluted at 1:400 With and Without Sulfanilamide. *J. Dairy Sci.*, 31: 817-822. 1948.
- (10) SALISBURY, G. W., FULLER, H. K., AND WILLETT, E. L. Preservation of Bovine Spermatozoa in Yolk-Citrate Diluent and Field Results from its Use. *J. Dairy Sci.*, 24: 905-910. 1941.
- (11) SALISBURY, G. W., AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent Upon Fertility of Bull Semen. *J. Dairy Sci.*, 30: 361-369. 1947.
- (12) SALISBURY, G. W., KNOTT, C. B., AND BRATTON, R. W. The Freezing Point Depression of Bull Semen and its Relation to the Diluter Problem. *J. Animal Sci.*, 7: 283-290. 1948.
- (13) SNEDECOR, G. W. Statistical Methods. 4th ed. The Collegiate Press, Inc. Ames, Iowa. 1946.
- (14) SWANSON, E. W. Varying the Proportion of Egg Yolk in Diluters for Bull Semen. *J. Dairy Sci.*, 31: 680. 1948.

FILLED MILKS FOR DAIRY CALVES. II. COMPARATIVE EFFECTS OF VARIOUS TYPES OF SOYBEAN OILS AND OF BUTTER OIL ON HEALTH, GROWTH AND CERTAIN BLOOD CONSTITUENTS¹

W. R. MURLEY, N. L. JACOBSON, G. H. WISE AND R. S. ALLEN

Iowa State College, Ames

Although the economic need for the substitution of relatively inexpensive oils and fats for milk fat in the diet of young calves has been recognized, vegetable oils generally have proved unsatisfactory for this purpose (5). Recent evidence (7) indicates that responses of calves fed a filled milk containing crude soybean oil are characterized by unthriftiness, excessive scouring and high mortality, but reactions of calves receiving hydrogenated soybean oil compare favorably with those of subjects fed milk fat. Since several distinct processing stages are involved in the preparation of hydrogenated soybean oil from the crude product, the relation of the accompanying alterations of the oil to its nutritional value warranted investigation.

The present study was designed to determine the effects of feeding various types of soybean oils to young calves on health and on the levels of certain constituents of the blood.

EXPERIMENTAL PROCEDURE

Twenty young calves, sixteen Holsteins and four Guernseys, were divided into four comparable groups of five animals each. From birth until 4 days of age all calves received mammary secretions from their respective dams. During the subsequent experimental period of 56 days, each calf was restricted to a diet of reconstituted milk supplemented with vitamins A and D and a mineral mixture. The daily dosage of vitamin A was 10,000 I.U. per 100 lb. body weight, whereas that of vitamin D was 1,000 I.U. per calf irrespective of size. A complex mineral mixture employed in a previous experiment (7) was given daily at the rate of 7 g. per 100 lb. body weight to eliminate possible complications from mineral deficiencies.

The distinguishing characteristic of the diets of the respective groups was the kind of oil or fat incorporated in the reconstituted milk. This product consisted of 10 per cent non-fat dry milk solids and 3 per cent oil or fat, dispersed in water by homogenization at 3,000 lb. pressure. On the basis of the source and the type of fat used, the dietary groups were as follows: Group I—milk fat, butter oil; Group II—soybean oil, crude; Group III—soybean oil, refined and bleached; Group IV—soybean oil, hydrogenated and deodorized. The butter oil was prepared by rendering high quality butter. The three soybean oils, representing major sequential stages in standard commercial processing, were from the same batch of oil.²

Received for publication March 8, 1949.

¹ Journal paper no. J-1462 of the Iowa Agricultural Experiment Station. Project no. 814.

² Supplied by Swift and Co., Chicago, Illinois.

The reconstituted milks, prepared immediately prior to each of the two daily feeding periods, were fed from nipple pails. Adjustments in feeding rates, based on weight changes of calves, were made at weekly intervals. The amount offered to normal calves daily was 1 lb. per 10 lb. body weight. Whenever evidences of digestive disturbances were detected, the amount fed was reduced by 50 per cent. This level was maintained until the health of the calf improved, after which the intake was increased gradually to the standard level.

Several special managemental and prophylactic measures were taken to minimize the number of possible complicating factors. All calves, housed in individual pens bedded with shavings, were muzzled to prevent excessive consumption of shavings and other extraneous materials. In view of the apparent susceptibility of young calves to gastro-enteric infections, "sulfathalidine"³ was administered at the rate of 6 g. daily per 100 lb. body weight during the first week of the experimental period (12).

Appraisal of the responses of the calves to the experimental treatments involved recorded observations of health, milk intake, weight changes, hemoglobin content of the blood and vitamin A, carotenoid and fat concentrations in the blood plasma. The calves were examined twice daily to detect any abnormalities, particularly diarrhea. The quantities of milk fed and refused at each feeding were measured accurately. At the beginning of the experimental period and at weekly intervals thereafter, calves were weighed and samples of venous blood were collected. The time of collection was 8 to 10 hours after the morning feeding and 20 to 22 hours after administration of vitamins. From each sample of blood, oxalated to prevent coagulation, 0.04 ml. was removed for hemoglobin determinations (9). The remainder was centrifuged within 24 hours to obtain plasma for further analyses. Vitamin A and carotene were determined by procedures described by Squibb *et al.* (13) and the fat by the method reported by Allen (1).

EXPERIMENTAL RESULTS

Health. In view of previous reports of high death losses of calves fed certain vegetable oils (5, 7) the mortality was surprisingly low; only one calf (group II) died during the course of the investigation. Since this animal survived to within 4 days of the termination of the trial, the missing items were supplied by computation (11).

Even though mortality was low, morbidity was high, particularly in groups receiving the crude and the refined soybean oils. The incidence of scours (table 1) was high in all groups during the first week the calves were on the experimental rations. Subsequently, the frequency of scours, though somewhat reduced, was erratic. During the entire trial, the incidence and the severity of the diarrhea were least in the calves fed butter oil, followed in order by the groups receiving, respectively, hydrogenated, refined and crude soybean oils. The differences in the dietary effects were manifested not only in the incidence of scours but also in the accompanying unthriftiness of the calves. Those in the crude and the refined soybean oil groups were thinner and more lethargic than the subjects in the

³ Provided by Sharp and Dohme, Glenolden, Pa.

other two groups. Differences in the health of animals receiving the butter oil and the hydrogenated soybean oil were not marked, but the degree of improvement in appearance as the feeding trial progressed was greater in the former group than in the latter.

Weight changes. Trends of the mean body weights for each group of calves, as shown in fig. 1, revealed approximately equal gains in calves fed reconstituted milks containing, respectively, butter oil (group I) and hydrogenated soybean oil, (group IV). A similar relationship was observed in calves receiving crude soy-

TABLE 1
Effect of type of oil in reconstituted milk diets of young calves on incidence of scouring

Dietary group	Calf. no.	Periodic (wk.) incidence of scourings ^a								
		1	2	3	4	5	6	7	8	8-wk. period
		(%)								
I (Butter oil)	3043				7.1			7.1	7.1	2.7
	3053									
	3056									
	3071	14.3			21.4					4.4
	3074	14.3	21.4							4.4
	Av.	5.7	4.3	0	5.7	0	0	1.4	1.4	2.3
II (Crude soybean oil)	3044					7.1		7.1	7.1	3.5
	3052	21.4	35.7	14.3	7.1				28.5	13.2
	3058	28.5	21.4	7.1	14.3		14.3	7.1	21.4	14.2
	3062 ^b	21.4		7.1					14.3	5.7
	3067	21.4	21.4	7.1	42.8	21.4	28.5	14.3		19.5
	Av.	18.5	15.7	7.1	12.8	5.7	8.6	5.7	14.3	11.2
III (Refined soybean oil)	3041	14.3					7.1	7.1		3.5
	3047	21.4	21.4	7.1	14.3	21.4	7.1	14.3	28.5	16.8
	3051					14.3	21.4	7.1	21.4	9.7
	3061	50.0				14.3	7.1	14.3	7.1	11.5
	3075		21.4	14.3	7.1		14.3	35.0	14.3	13.3
	Av.	17.1	8.5	4.3	4.3	10.0	11.4	15.6	14.3	11.0
IV (Hydrogenated soybean oil)	3039	21.4		35.0	7.1				7.1	8.0
	3040	21.4			14.3	21.4				6.2
	3048	28.5	14.3		14.3		7.1	7.1		8.9
	3063	21.4	7.1							3.5
	3072									
	Av.	18.5	4.3	7.0	7.1	4.3	1.4	1.4	1.4	5.3

^a Number of times scouring observed for the specific period ÷ number observations × 100.

^b Died 4 d. prior to termination of experimental period.

bean oil-filled milk (group II) and those fed refined soybean oil-filled milk (group III). Even though weight gains for groups I and IV were greater than those for groups II and III, the differences were not significant statistically. This lack of significance is ascribable, in part, to the marked intra-group variations. The ranges of weight changes, in pounds, for the respective groups were: group I, 18 to 37; group II, -9 to 28; group III, -2 to 34 and group IV, 11 to 49.

Ratios of milk ingested to weight gains. The ratios were erratic, inasmuch as the quantities of milk consumed and the body weight changes within the different groups of calves were highly variable. However, the ratios of milk consumed to weight increases (table 2) for the individual groups revealed, on one extreme, a marked similarity between the groups of calves receiving the butter oil

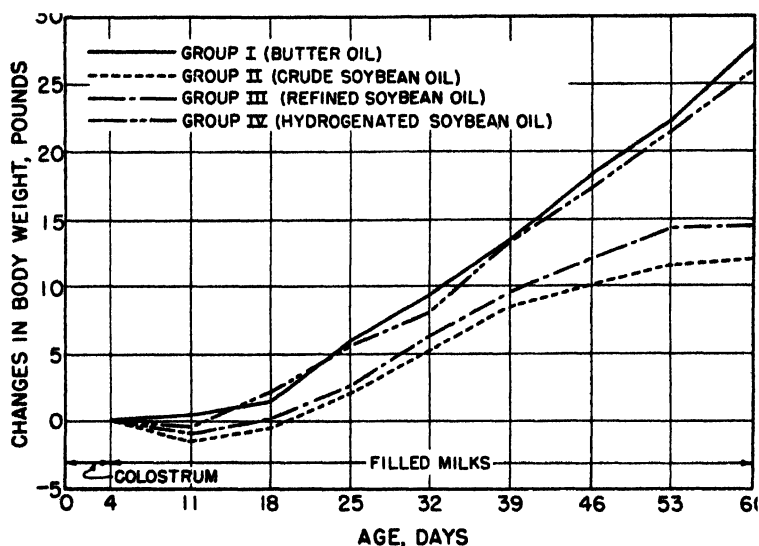


FIG. 1. Effect of type of oil in the reconstituted milk diet of calves on the changes in body weights.

and the hydrogenated soybean oil and, on the other, a close resemblance between the groups of animals given crude and refined soybean oils. The range of ratios, from 1:16.1 to 1:31.2, is striking.

Blood constituents. a. Hemoglobin of blood. The hemoglobin values presented in table 3 reveal marked variability among individuals but similarity in

TABLE 2
Effect of type of oil in the milk fed to calves on the ratio of quantity ingested to the gain in weight

Group	Oil in diet	Av. milk consumed (lb.)	Av. wt. gains (lb.)	Milk/lb. gain (lb.)
I	Butter	450.7	28.0	16.1
II	Crude soybean	405.2	13.0	31.2
III	Refined soybean	449.0	14.6	30.8
IV	Hydrogenated soybean	468.8	26.0	18.0

means for the groups. Since only one calf, 3039 of group IV, had hemoglobin levels sufficiently low to be considered in the anemic range, a deficiency of hemopoietic constituents apparently did not contribute to differences in the general health of calves in the various dietary groups. There was, however, in all groups

a slight decline in concentrations from the early stages of the trial to approximately the sixth week.

b. Vitamin A and carotenoids of blood plasma. The data relating to these constituents are shown in tables 4 and 5. If 10 γ of vitamin A per 100 ml. of blood plasma be accepted as the lower level of the normal range of concentrations (3), there were deficient calves in each group (table 4). In accordance with expectations, the extent of the deficiency was least evident in the calves receiving butter oil, inasmuch as this fat contained 5.50 γ of vitamin A and 4.42 γ of caro-

TABLE 3
Concentration of hemoglobin in the blood of calves receiving different oils in the reconstituted milk diets

Dietary group	Calf no.	Concentrations of hemoglobin at 7-d. intervals								
		4	11	18	25	32	39	46	53	60
		(g./100 ml.)								
I (Butter oil)	3043					9.8	7.9	9.5	8.8	8.6
	3053	11.3	13.9	13.3	12.6	10.2	11.6	8.6	10.3	9.7
	3056	12.5	11.5	10.5	10.3	10.0	9.5	9.3	8.8	9.5
	3071	16.9	14.2	11.6	11.6	12.1	11.5	12.7	12.1	13.1
	3074	15.0	14.9	13.0	13.2	13.0	11.9	10.8	11.3	11.4
	Av.	13.9	13.6	12.1	11.9	11.3 ^a	11.1 ^a	10.3 ^a	10.6 ^a	10.9 ^a
II (Crude soybean oil)	3044				9.7	9.7	13.3	12.4	11.3	11.6
	3052	13.7	19.3	15.1	15.0	12.5	12.4	9.9	9.6	9.9
	3058	15.0	14.7	13.9	13.1	11.8	12.9	10.4	11.0	11.3
	3062	13.7	13.3	12.4	10.9	9.9	10.4	11.3	11.6	11.6
	3067	14.5	11.9	13.2	12.3	11.8	10.9	11.0	11.1	9.7
	Av.	14.2	14.8	13.7	12.8 ^a	11.5 ^a	11.7 ^a	10.7 ^a	10.8 ^a	10.6 ^a
III (Refined soybean oil)	3041					12.5	10.2	10.9	11.3	10.6
	3047	15.0	15.0	11.1	14.7	11.6	11.5	11.1	11.6	14.5
	3051	11.9	14.3	15.0	13.1	12.0	12.7	11.1	11.0	10.5
	3061	16.9	16.3	15.5	14.8	12.4	10.7	11.1	11.0	10.1
	3075	13.4	13.3	12.5	12.1	13.4	11.6	9.4	9.7	8.5
	Av.	14.3	14.7	13.5	13.7	12.3 ^a	11.6 ^a	10.7 ^a	10.8 ^a	10.9 ^a
IV (Hydrogenated soybean oil)	3039						7.9	6.6	9.1	7.1
	3040						8.9	10.0	12.3	10.3
	3048	15.0	18.1	11.2	12.6	12.5	10.9	11.9	11.5	11.6
	3063	13.9	14.5	13.9	12.7	11.2	10.3	11.8	9.6	13.5
	3072	12.0	11.9	10.3	9.8	11.1	9.1	8.8	7.7	9.1
	Av.	13.6	14.8	11.8	11.7	11.6	10.1 ^a	10.8 ^a	9.6 ^a	11.4 ^a

^a Only calves on which hemoglobin measurements were made throughout included in the av.

tene per g., whereas the vegetable oils probably were almost devoid of vitamin A activity. Among the calves fed soybean oils, the vitamin A values were lower in the subjects fed the milks containing the crude and the refined oils than in those receiving the hydrogenated oil. These inter-group differences, however, were not statistically significant.

Throughout the trial, the carotenoid concentrations in the blood plasma also were extremely variable among individual calves (table 5). The highest values

were in plasma from Guernsey calf 3058, receiving crude soybean oil. Mean levels of the carotenoids for the groups of calves fed butter oil and crude soybean oil were similar and higher than for the other groups. The lowest values were observed in group IV (hydrogenated soybean oil). Though the differences between the highest and the lowest group means are great they are not significant statistically, inasmuch as the number of animals in each group was small and the variability great.

TABLE 4

Effect of type of oil in reconstituted milk diets of young calves on concentrations of vitamin A in blood plasma

Dietary group	Calf no.	Vitamin A levels at 7-d. intervals								
		4	11	18	25	32	39	46	53	60
I (Butter oil)		(γ/100 ml.)								
	3043	13.0	27.4	23.2	17.4	16.0	16.0	14.5	17.0	15.8
	3053	18.2	14.7	12.7	16.3	11.5	16.7	15.5	15.7	9.5
	3056	16.7	15.0	18.0	18.3	20.1	16.7	10.8	10.7	10.7
	3071	13.6	10.3	14.7	8.1	5.5	7.5	9.6	15.9	15.3
	3074	25.0	28.0	21.7	7.9	10.2	12.4	13.4	13.9	15.5
	Av.	17.3	19.1	18.1	13.6	12.7	13.9	12.8	14.6	13.4
II (Crude soybean oil)	3044	19.9	14.1	12.2	6.5	10.9	7.9	11.1	8.6	8.1
	3052	20.4	13.1	8.9	13.0	10.3	11.4	18.0	11.1	8.8
	3058	11.5	8.0	12.5	13.3	12.2	8.4	6.5	2.0	5.8
	3062	20.0	18.7	10.8	10.0	6.2	2.3	1.9	3.4	4.9*
	3067	15.6	11.9	13.8	6.4	4.7	11.7	8.8	5.0	2.8
	Av.	17.5	13.2	11.6	9.8	8.8	8.3	9.3	6.0	6.1
III (Refined soybean oil)	3041	21.1	17.4	10.2	6.8	7.7	9.9	6.0	11.1	13.1
	3047	16.6	6.7	9.5	6.8	7.7	8.0	5.3	8.8	10.0
	3051	15.7	13.0	11.7	10.6	9.6	10.3	9.6	6.5	3.7
	3061	19.3	14.0	25.1	9.3	5.3	3.4	7.6	6.1	6.0
	3075	24.5	14.0	5.2	10.9	6.4	7.1	10.8	7.0	3.8
	Av.	19.4	13.0	12.3	8.9	7.3	7.7	7.9	7.9	7.3
IV (Hydrogenated soybean oil)	3039	22.0	10.1	16.7	14.5	7.5	14.2	12.4	9.4	11.2
	3040	13.3	19.7	13.7	11.2	9.6	12.9	13.8	13.8	8.3
	3048	12.5	5.8	8.6	9.6	9.4	9.5	11.2	8.7	12.3
	3063	22.2	23.3	15.1	15.2	9.2	6.6	2.2	8.8	5.3
	3072	17.1	18.6	15.8	8.5	6.7	12.3	7.7	9.9	6.8
	Av.	17.4	15.5	14.0	11.8	8.5	11.1	9.5	10.1	8.8

* Calf died. Item supplied by missing data formula.

c. *Fat concentrations in blood plasma.* Blood plasma fat levels (table 6) decreased in all groups during the first week after changing from colostral secretions to the respective reconstituted milks. Subsequently, the trends were upward, attaining the maximum the third week. The changes thereafter were irregular, particularly in the plasma from calves receiving the soybean oils. The plasma fat content was significantly higher in the groups of calves fed the crude and the refined soybean oils than in those receiving the other oils. During the latter half of the trial, plasma fat levels for the calves receiving butter oil were

over twice as high as those for calves receiving the hydrogenated oil, a difference which was highly significant statistically.

DISCUSSION

In accordance with previous observations (7), the health of calves fed filled milk containing hydrogenated soybean oil was markedly similar to that of calves given a reconstituted milk containing butter oil. In contrast to the relatively good physical condition of animals in these groups, diarrhea, low weight gains and

TABLE 5

Effect of type of oil in reconstituted milk diets of young calves on concentrations of carotenoids in blood plasma

Dietary group	Calf no.	Carotenoids levels at 7-d. intervals									
		11	18	25	32	39	46	53	60		
I (Butter oil)				(γ/100 ml.)							
	3043	21.3	9.8	20.2	19.4	6.8	6.2	9.8	9.8	8.5	
	3053	7.9	10.7	14.3	11.7	21.1	23.4	16.8	17.2	14.3	
	3056	28.8	13.2	13.6	27.5	21.7	11.5	12.2	7.7	9.8	
	3071	3.4	4.0	2.8	5.8	10.2	9.4	9.6	17.1	22.2	
	3074	13.2	1.9	3.4	7.7	9.4	12.4	8.1	12.3	15.1	
	Av.	14.9	7.9	10.9	14.4	13.8	12.6	11.3	12.8	14.0	
II (Crude soybean oil)	3044	18.5	26.2	21.9	13.2	12.3	15.5	16.2	15.8	15.3	
	3052	12.2	11.5	5.5	7.0	11.9	11.7	10.7	9.8	3.0	
	3058	25.3	13.4	30.3	43.0	41.3	43.7	24.3	23.7	23.7	
	3062	12.1	6.8	9.8	10.4	6.4	4.0	2.5	2.6	2.1 ^a	
	3067	11.3	11.5	10.9	7.7	8.5	9.4	10.7	5.0	6.4	
	Av.	15.9	13.9	15.7	16.3	16.1	16.9	12.9	11.4	10.1	
	III (Refined soybean oil)	3041	19.4	19.4	14.5	9.8	6.2	6.4	5.8	5.5	8.5
3047		43.0	8.5	11.9	12.6	13.0	10.2	5.7	9.2	7.5	
3051		12.1	10.0	12.3	8.7	13.0	9.8	11.5	6.6	3.4	
3061		22.6	7.2	13.8	8.9	4.3	2.6	0.9	2.5	2.3	
3075		13.4	7.5	4.9	5.5	3.4	7.1	2.5	2.1	0.4	
Av.		22.1	10.5	11.5	9.1	8.0	7.2	5.3	5.2	4.4	
IV (Hydrogenated soybean oil)		3039	95.7	23.0	25.2	19.2	9.2	3.6	4.5	3.8	2.6
	3040	20.0	10.9	8.7	5.1	4.2	1.7	2.8	5.3	3.2	
	3048	6.6	3.6	1.0	0.8	1.0	3.4	1.5	2.1	5.1	
	3063	19.6	7.2	6.0	4.5	1.9	4.7	6.2	0.8	1.9	
	3072	24.9	8.7	5.1	3.4	5.3	2.5	7.7	0.0	3.2	
	Av.	33.4	10.7	9.2	6.6	4.3	3.2	4.5	2.4	3.2	

^a Calf died. Item supplied by missing data formula.

high morbidity prevailed among subjects fed the crude and the refined soybean oils. These unsatisfactory responses corroborate previous findings (5, 7), except that the mortality was lower and the general health was somewhat better than reported earlier. These apparent discrepancies are tenable, inasmuch as the duration of the experiment reported herein was shorter than that of Gullickson *et al.* (5) and, contrary to the procedure followed by Jacobson *et al.* (7), milk intake by diarrhetic calves was reduced, thus tending to alleviate the undesirable

reactions to the diet. Moreover, it is possible that the soybean oils used in the various investigations were dissimilar in quality and composition.

The largest weight increases were made by calves receiving the butter oil and the hydrogenated soybean oil, but even these gains were substandard (10). This retarded growth evidently was due largely to an inadequate intake of total digestible nutrients, which was approximately 25 per cent less than the recommended allowance (8). In addition to the effect of the low energy content of

TABLE 6
*Effect of type of oil in reconstituted milk diets of young calves
on concentrations of fat in blood plasma*

Dietary group	Calf no.	Fat concentrations at 7-d. intervals								
		4	11	18	25	32	39	46	53	60
I (Butter oil)		(mg./100 ml.)								
	3043	100	58	142	156	114	114	127	66	94
	3053	79	64	98	146	153	159	144	122	124
	3056	113	73	125	152	138	103	118	126	164
	3071	66	23	98	115	108	126	169	179	184
	3074	79	19	117	168	138	98	134	182	157
	Av.	87.4	47.4	116.0	147.4	132.2	120.0	138.4	135.0	144.6
II (Crude soybean oil)	3044	102	136	202	194	217	236	231	219	244
	3052	98	31	34	178	146	185	158	110	69
	3058	39	64	167	294	280	126	231	188	222
	3062	62	66	162	163	167	117	128	139	141 ^a
	3067	111	85	164	204	162	191	205	167	157
	Av.	82.4	76.4	145.8	206.6	194.4	171.0	191.0	164.6	166.6
	III (Refined soybean oil)	3041	104	118	198	236	244	319	217	213
3047		124	26	105	182	151	141	154	127	177
3051		142	135	200	377	331	318	239	233	263
3061		118	56	188	163	226	194	234	233	206
3075		75	78	154	208	193	174	131	125	121
Av.		112.6	82.6	169.0	233.2	229.0	229.2	195.0	186.2	185.4
IV (Hydrogenated soybean oil)		3039	114	16	61	44	31	62	51	46
	3040	110	28	78	106	128	43	60	64	44
	3048	66	19	24	30	8	34	68	54	76
	3063	126	62	112	95	83	86	89	78	50
	3072	148	28	85	97	73	55	50	60	62
	Av.	112.8	30.6	72.0	74.4	64.6	56.0	63.6	60.4	51.2

^a Calf died. Item supplied by missing data formula.

the rations, the poor growth of the calves in the crude and the refined soybean oil groups undoubtedly was associated with frequency and severity of scouring.

The small weight gains per unit of milk ingested by calves fed the crude and the refined soybean oils may be attributed primarily to the high incidence of digestive disturbances and the concomitant reduction in milk consumption. The rapid passage of ingesta through the digestive tract during periods of diarrhea probably affected adversely the efficiency of absorption.

The tendency of the hemoglobin values to decline during the early stages of

the experiment is in accord with observations on calves receiving normal diets (15). If the initial hemoglobin decrease is of dietary etiology, the mineral mixture evidently was inadequate to maintain the high concentrations. Since the levels in individual calves were, in most instances, within the normal range, anemia apparently was not a complicating factor.

Inasmuch as differences in blood plasma vitamin A levels among the groups of calves fed the various soybean oils were not marked, this vitamin evidently was not a primary factor in the differences in the response of young calves to these oils. It is significant, however, that blood plasma vitamin A levels of all calves fed the soybean oil rations were extremely low. None of the animals in these three groups consistently maintained values above 10 γ per 100 ml. of blood plasma, the level recommended for adequate vitamin A nutrition (3). These low concentrations indicate that under the conditions of this experiment young calves need a daily vitamin A intake greater than 10,000 I. U. per 100 lb. body weight.

The calves fed butter oil had a daily intake of approximately 14,000 I. U. of vitamin A activity per 100 lb. body weight, yet the plasma vitamin A levels were low. The values for three calves in this group were within the deficiency range during a portion of the experimental period.

In contrast to the findings of Squibb *et al.* (13) in experiments conducted with lactating cows, inclusion of crude soybean oil in the ration of young calves did not depress blood plasma carotenoid levels. Conversely, after the first week, the mean values for the group receiving crude soybean oil were considerably higher than those for the calves fed the refined and the hydrogenated soybean oils and, except for the last 2 weeks, slightly higher than those of the calves receiving butter oil. Although the differences among groups were not significant, the fact that the carotenoid values were high for certain individuals of the crude oil group suggests that the oil supplied a fat-soluble pigment similar to carotene. Comparative spectrophotometric studies of extracts from butter oil and crude soybean oil further indicated similarity of the pigments. No vitamin A activity of the soybean oil pigment, however, was reflected in the blood plasma vitamin A concentrations.

Blood plasma carotenoid and vitamin A levels were reduced when calves scoured, a relationship observed by other investigators (6, 14), but apparently were unaffected by abnormally high body temperatures.

The marked decline in blood plasma fat values of all groups during the first week of the experiment may be attributed in part to the high incidence of scouring, which probably resulted in a low coefficient of absorption during this period. Another factor that might have been involved was the possible reduction in daily fat intake at the time the calves were transferred from colostrum to the experimental rations.

The comparatively high blood plasma fat values for the crude and refined soybean oil groups are similar to observations made by Gullickson *et al.* (5) on older calves fed corn and cottonseed oils. Although there is no obvious reason

for the differences in the fat levels of calves fed the various oils, possible dissimilarities in the rate and the extent of absorption and in the subsequent metabolism of the oils merit consideration.

The characteristics of crude soybean oil causing the deleterious effects on young calves apparently are eliminated in the preparation of the hydrogenated and deodorized oil from the crude product. Since refining and bleaching failed to improve the value of the oil for calf feeding, evidently the free fatty acids, phosphatides, other non-glyceride constituents and pigments removed in the processing did not contribute to the ill effects resulting from feeding crude oil. Therefore, modifications induced by hydrogenation and/or by related processes seemingly enhance the nutritive value of soybean oil.

Graham and Cupps (4) found that hydrogenated herring oil is less toxic than the unhydrogenated product when fed to goats. This observation prompted the suggestion that the arrangement of the double bonds in the oil may be a factor involved in the physiological reaction. Moreover, it is possible that some substance toxic to the young calf may be removed or inactivated during deodorization (2). The obscure nature of the relationships between the physiological reactions of the calf and the physical and chemical characteristics of the dietary fat indicates a need for further investigation.

SUMMARY

Three types of soybean oils, crude, refined and hydrogenated, were compared with butter oil as components of reconstituted milk rations for dairy calves during the period from 4 to 60 days of age. The respective milks, supplemented with a mineral mixture and vitamins A and D, were fed to four comparable groups of calves.

Although there was considerable intra-group variation, the incidence of scouring was lowest for the calves fed butter oil, followed in order by the groups receiving, respectively, hydrogenated, refined and crude soybean oils. Frequent and severe diarrhea was accompanied by unthriftiness and lethargy.

The mean weight gains of the calves fed hydrogenated soybean oil were similar to those of the group receiving butter oil but greater than the mean weight gains of the groups fed the refined and the crude soybean oils, but the differences were not significant statistically.

There were no appreciable differences in the mean hemoglobin levels among the various groups.

Since there were no significant differences in the mean plasma vitamin A values among the groups of calves fed the various soybean oils, the differences in growth and in state of health cannot be attributed to the level of vitamin A intake.

Although the mean carotene levels of the blood plasma of the groups fed the butter oil and the crude soybean oil were considerably higher than those of the other groups, the differences were not significant statistically.

Mean blood plasma fat levels for the calves receiving butter oil were significantly higher than those of the group fed hydrogenated soybean oil but significantly lower than the mean values of the other two groups.

ACKNOWLEDGMENTS

The authors are grateful to Dr. S. F. Scheidy, Sharp and Dohme, Inc., Glenolden, Pa., for supplying the "sulfathalidine"; to Dr. E. W. Bird, Department of Dairy Industry, Iowa State College, for analyzing the butter oil for vitamin A and carotene; and to Prof. O. Kempthorne, Department of Statistics, Iowa State College, for suggestions in statistical analysis of the data.

REFERENCES

- (1) ALLEN, N. N. Blood Fat of Dairy Cattle. 1. A Simple Volumetric Method for Determining Blood Fat. 2. Factors Influencing the Fat Content of Blood Plasma. Minn. Agr. Expt. Sta. Tech. Bull. 130. 1938.
- (2) BALDWIN, A. R. Effects of Deodorization on the Stability of Vegetable Oils. J. Am. Oil Chemists' Soc., 25: 33-35. 1948.
- (3) BOYER, P. D., PHILLIPS, P. H., LUNDQUIST, N. S., JENSEN, C. W., AND RUPEL, I. W. Vitamin A and Carotene Requirements for the Maintenance of Adequate Blood Plasma Vitamin A in the Dairy Calf. J. Dairy Sci., 25: 433-439. 1942.
- (4) GRAHAM, W. R., JR., AND CUPPS, P. T. The Action of Herring Oil before and after Hydrogenation on the Yield and Fat Percentage of the Milk of the Goat. J. Dairy Sci., 21: 45-48. 1938.
- (5) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves. J. Dairy Sci., 25: 117-128. 1942.
- (6) HIBBS, J. W., AND KRAUSS, W. E. The Effect of Feeding Vitamin A on the Blood Picture and on Liver Storage in Calves. J. Dairy Sci., 29: 519-520. 1946.
- (7) JACOBSON, N. L., CANNON, C. Y., AND THOMAS, B. H. Filled Milks for Dairy Calves. I. Soybean Oil Versus Milk Fat. J. Dairy Sci., in press. 1949.
- (8) LOOSLI, J. K., HUFFMAN, C. F., PETERSEN, W. E., AND PHILLIPS, P. H. Recommended Nutrient Allowances for Domestic Animals. No. III. Recommended Nutrient Allowances for Dairy Cattle. Natl. Research Council, Washington, D. C. 1944.
- (9) NEWCOMER, H. S. Absorption Spectra of Acid Hematin, Oxyhemoglobin, and Carbon Monoxide Hemoglobin. J. Biol. Chem., 37: 465-496. 1919.
- (10) RAGSDALE, A. C. Growth Standards for Dairy Cattle. Mo. Agr. Expt. Sta. Bull. 336. 1934.
- (11) SNEDECOR, G. W. Statistical Methods. 4th ed. Iowa State College Press, Ames. P. 268. 1946.
- (12) SPIELMAN, A. A., LOOSLI, J. K., THOMAS, J. W., NORTON, C. L., AND TURK, K. L. Carotene Utilization in the Newborn Calf. J. Dairy Sci., 29: 381-391. 1946.
- (13) SQUIBB, R. L., CANNON, C. Y., AND ALLEN, R. S. Effect of Raw Soybeans and of Soybean Oil on Plasma Carotene and on Vitamin A as Measured by Activated Glycerol Dichlorohydrin. J. Dairy Sci., 31: 421-427. 1948.
- (14) SUTTON, T. S., AND KAESER, H. E. Some Physiological Effects of Extending the Colostrum Feeding Period of Dairy Calves. J. Dairy Sci., 29: 13-26. 1946.
- (15) WISE, G. H., CALDWELL, M. J., PARRISH, D. B., FLIPSE, R. J., AND HUGHES, J. S. Changes in Cell Volume and in Concentration of Hemoglobin and of Several Inorganic Constituents of the Blood of Calves during Early Postnatal Development. J. Dairy Sci., 30: 983-993. 1947.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

JULY, 1949

NUMBER 7

COMPARISON OF METHODS OF ESTIMATING MILK AND FAT PRODUCTION IN DAIRY COWS

M. H. ALEXANDER AND W. W. YAPP

Department of Dairy Science, University of Illinois, Urbana

Production records of milk and fat constitute an important and necessary part of the program of herd improvement in dairy cows. In Illinois there are at present about 1,123,000 dairy cows of milking age, of which less than 4 per cent are being tested for production. This is too small a number to afford a satisfactory basis for any widespread improvement.

Cost may be a limiting factor in restricting the scope of testing. This is suggested by the fact that in Illinois in 1936 there were 29 herds, including 326 cows, on Advanced Registry test. The numbers on this type of test had in-

TABLE 1
*Approximate cost per year in Illinois for testing by three
different types of tests, 1947**

Type of test	Cost/cow	Cost/herd	Av. no. cows /herd
A. R.	\$22.60	\$293.80	13
H. I. R.	8.65	181.65	21
D. H. I. A.	3.65	84.00	23

* Derived from Illinois testing fee scales for 1947.

creased by 1945 to 48 herds and 652 cows. In 1936 there were in Illinois 27 herds, including 619 cows, in Herd Improvement Registry testing. By 1945, this number had increased to 165 herds including 3,586 cows. In 1936 there were 23,812 cows tested in Illinois in Dairy Herd Improvement Associations. This figure rose to 44,282 in 1941. The number declined seriously during the war and is not yet back to the 1941 peak. Table 1 gives the comparative costs of the three different types of testing.

This investigation was carried out to devise a method of testing that will lessen the amount of time and labor required on the part of the tester and the cow owner, and so to spread testing efforts over a wider area.

The semi-official test, the test used in this study for the standard or measure of reliability of other tests, either now in use or proposed, was adopted by the various breed associations as a sufficiently accurate method of measuring produc-

Received for publication August 5, 1948.

tion. It departed from the method of the official test by the device of using only a 2-day test period each month together with daily milk weights. Later the test period was reduced to 1 day per month. Later still the bi-monthly 1-day test period was adopted as optional with the producer. The results obtained by this departure from the strictly official method of supervision of tests encourages the belief that some further departure from the present methods of testing may yield sufficiently satisfactory results to merit consideration.

REVIEW OF LITERATURE

A 1-day test every 30 days has yielded results within 2 per cent of those obtained by testing every day (6). Later it was found (7) that a 1-day bi-monthly test varied only 3.8 per cent from the daily test. Bi-monthly tests (8) were found to be practically as accurate as monthly tests, having a coefficient of correlation of 0.97 with the standard error of estimate within the limit of significance.

In methods of testing involving less frequent test periods, a high correlation was observed between a 1-day test taken during the fifth month (1, 3) and the monthly method of testing. The correlation between the 7-day test (12), taken at the beginning of the lactation period, and the yearly test was low.

The monthly decrease in production was found to be very uniform in all breeds (10), the production in the twelfth month being approximately 50 per cent that of the best month.

Comparing the monthly to the bi-monthly method of testing. McDowell (7), working with 70 C.T.A. records, found that the monthly and bimonthly test varied on the average 2.91 and 3.80 per cent, respectively, from the actual. The greatest variation for the C.T.A. method was 3.8 per cent, as compared to 12.5 per cent for the bi-monthly method. Dairymen estimating production in 48 cases erred by differences of from 1 to 63 per cent with an average error of 28.0 per cent from the C.T.A. record.

Gifford (4) found that 69.0 per cent of more than 100 Advanced Registry Holstein and Guernsey records were within the standard error of estimate comparing the monthly with the bi-monthly test plan, 95.0 per cent were within two times the standard error of estimate and 99.17 per cent were within three times the standard error of estimate, the standard error of estimate in each case being sufficiently small to make this finding significant. The coefficients of correlation between monthly and bi-monthly testing were high. He concluded that the bi-monthly testing is a satisfactory method of estimating production.

McKellip and Seath (8), in comparing bi-monthly to monthly records, found that the coefficient of correlation was over 0.97, and the standard error of estimate within the limit of significance. They concluded that bi-monthly tests when used with daily milk weights were practically as accurate as monthly records made by centering the tests and not using daily milk weights.

Gifford (4), using 841 records divided into four groups according to various production levels, found coefficients of correlation ranging from 0.956 to 0.997

between the different groups, and between assorted pairs of the various groups, differences in the coefficients ranging from 0.001 to 0.015. He concluded that bi-monthly testing is satisfactory.

An analysis of 500 Jersey records (2) comparing the monthly to the bi-monthly plan with 250 of the records made under 2-day supervision and 250 under 1-day supervision showed that 258 of the bi-monthly records exceeded the monthly records and 242 were lower. The average variation for all records was 7.21 lb. of fat.

Predicting yearly yields from short time records. Petersen (9) comparing the 1- and 2-day tests on 35 Jerseys, found that all but five cows varied less than 2 per cent and only one cow varied as much as 3 per cent.

Cannon *et al.* (1) found that, based on a single 1-day test and using 400 Advanced Registry records and 1,289 Dairy Herd Improvement Association records, prediction factors for production were determined which showed a high correlation between estimated yield and actual yield. It was found that a 1-day test taken during the fifth month gave the most dependable prediction of yield.

Yapp (12), studying the reliability of the 7-day test, showed that the correlation between the 7-day test and the yearly test was not high and concluded that the 7-day test is not a satisfactory criterion of semi-official production.

Gaines (3) found that a 7-day test made shortly after calving did not give a true indication of the whole lactation but that such a test conducted 60 days or more after calving was more accurate. He found that a 7-day test conducted during the fifth month most nearly indicated the lactation record.

Turner (10) found that the monthly decrease in production is very uniform in all breeds, the production in the twelfth month being approximately 50 per cent of that of the best month. He concluded that the principal causes for variation are nutrition and temperature.

EXPERIMENTAL PROCEDURE

The milk and butterfat records made on the five major dairy breeds kept in the herd at the Illinois Station were used as a source of data for this study. Since 1932 all of the cows in the various breeds have been kept under as nearly the same environmental conditions as possible, thus minimizing one important variable. All records of less than 305 days were discarded. All records of more than 305 days that showed plain indications of abnormalities, such as abortion, also were discarded. (The records included only the first 305 days of the lactation period.) This was called the semi-official record, since the test was made by the semi-official method using a 1-day supervision period. The semi-official record for 305 days then was used as the standard against which the other methods developed in this study were measured. Six hundred and eighty-four records meeting these conditions were found and these form the basis of the material used in this study.

Different methods studied. Since the semi-official test was used as the standard and since it seemed desirable to make as direct comparisons as possible, it

TABLE 2

Variation from semi-official, or actual, of four different methods of estimating production of fat-corrected milk

Breeds	Total no. of cows	Method I Bi-monthly test				Method II Tests taken 2nd 6th and 10th mo.				Method III Tests taken 2nd and 10th mo.				Method IV Tests taken 4th and 8th mo.			
		Cows varying from actual records															
		0-2.0 %	2.1- 5.0%	5.1- 10.0 %	Above 10.1 %	0-2.0 %	2.1- 5.0%	5.1- 10.0 %	Above 10.1 %	0-2.0 %	2.1- 5.0%	5.1- 10.0 %	Above 10.1 %	0-2.0 %	2.1- 5.0%	5.1- 10.0 %	Above 10.1 %
All	684	% 43.27	29.54	20.03	7.16	42.40	28.95	20.91	7.74	33.76	27.33	26.50	12.41	28.65	27.92	28.95	14.47
	No.	296	202	137	49	290	198	143	53	231	187	182	84	196	191	198	99
Ayrshire	44	% 31.81	31.81	15.95	20.43	29.54	31.83	9.09	29.54	18.18	18.18	31.82	31.82	18.18	25.00	40.91	15.91
	No.	14	14	7	9	13	14	4	13	8	8	14	14	8	11	18	7
Brown Swiss	89	% 39.33	39.33	16.85	4.49	41.57	34.83	17.98	5.62	31.46	38.71	29.21	5.62	31.46	26.97	26.97	14.60
	No.	35	35	15	4	37	31	16	5	28	30	26	5	28	24	24	13
Guernsey	87	% 35.63	27.58	27.58	9.21	29.88	36.78	26.44	6.90	36.78	21.84	25.29	16.09	21.84	25.29	33.33	19.54
	No.	31	24	24	8	26	32	23	6	32	19	22	14	19	22	29	17
Holstein	382	% 48.69	27.48	18.32	5.51	47.12	26.70	20.16	6.02	36.13	28.01	25.92	9.94	31.68	28.80	28.01	11.51
	No.	186	105	70	21	180	102	77	23	138	107	99	38	121	110	107	44
Jersey	82	% 36.58	29.27	25.61	8.54	41.46	24.39	26.83	7.32	30.49	28.05	25.61	15.85	24.39	29.27	24.39	21.95
	No.	30	24	21	7	34	20	22	6	25	23	21	13	20	24	20	18

was decided to use different months from these same records as a basis for computation of the new methods.

The methods used in this study for estimating production of milk and butterfat were as follows: Method I, wherein the tests are taken every other month (bi-monthly); method II, wherein the tests are taken on the second, sixth and tenth months; method III, wherein the tests are taken on the second and tenth months; and method IV, wherein the tests are taken on the fourth and eighth months. The formulas used for estimating yields for the various methods were as follows¹: Method I - $Y = (61m_1) + (61m_3) + (61m_5) + (61m_7) + (61m_9)$; method II - $Y = (102m_2) + (102m_6) + (101m_{10})$; method III - $Y = (153m_2) + (152m_{10})$; Method IV - $Y = (153m_4) + (152m_8)$.

TABLE 3
*Comparison of the accuracy of five different methods of testing
(684 records of fat-corrected milk, all breeds)*

	Semi-official	Method I	Method II	Method III	Method IV
Av. of plus parameters (<i>lb.</i>)	15,573	15,199	15,327	15,531	15,197
Av. of minus parameters (<i>lb.</i>)	8,215	7,707	7,797	7,891	7,569
Records above plus parameters (%)	12.0	12.3	11.5	12.4	11.3
Records below minus parameters (%)	9.5	8.5	9.4	9.9	7.4
Variation of plus parameters from semi-official parameters (%)	0.0	2.5	1.8	0.3	2.5
Variation of minus parameters from semi-official parameters (%)	0.0	6.2	5.1	3.9	7.8

In no case was any test used that was taken earlier than the first full month of lactation. Except for the estimate on the standard or semi-official yield, none of these methods can be considered as being based on the centering method of testing. However, since estimates I, II, III and IV were derived from different testing periods of the semi-official records, which were centered, they do derive some of whatever benefits may accrue to the centering method, and to that extent are exempt from any criticism that may arise from the use of different systems.

Various other groups of test periods within the standard records used were tried. The fact that none of these combinations was as accurate in estimating the standard yield as the four groups selected caused them to be discarded. These four methods were compared to the standard method for their accuracy in estimating milk, butterfat and FCM yields. Their standard deviations, coefficients of correlation, coefficients of variation and coefficients of regression were determined as measures of comparison to the standard method.

¹ Y = yield for 305 days; m indicates test for month in which test was made.

As has been noted previously (2, 4, 7), the bi-monthly method of testing has been found to be as accurate for all practical purposes as testing only once a month. This study shows that bi-monthly testing yielded results with a high degree of dependability when the 684 records included in the study were compared on the bi-monthly basis with the same records considered on the monthly

TABLE 4

Percentage of error groups for four different types of tests, showing variation from semi-official test in terms of fat-corrected milk

Breed	Type of test	Cows varying from mean S.O.				
		1-5%	1-10%	1-15%	1-20%	More than 20%
		(%)	(%)	(%)	(%)	(%)
Ayrshire	S.O.	20	34	39	66	34
	I	20	30	45	64	36
	II	20	30	45	70	30
	III	11	27	45	60	40
	IV	9	34	45	63	37
Brown Swiss	S.O.	11-	31	52	63	37
	I	13	30	46	64	36
	II	17	34	43	62	38
	III	13	29	47	60	40
	IV	10	20	45	60	40
Guernsey	S.O.	17	48	66	77	23
	I	20	42	61	76	24
	II	24	39	64	75	25
	III	22	40	58	70	30
	IV	25	39	56	77	23
Jersey	S.O.	20	41	51	73	27
	I	21	41	59	77	23
	II	21	38	54	77	23
	III	20	35	51	68	32
	IV	23	43	61	74	26
Holstein-Friesian	S.O.	16	35	52	68	32
	I	21	33	50	68	32
	II	21	34	48	67	33
	III	17	31	49	65	35
	IV	19	35	52	67	33
All breeds	S.O.	14	28	44	56	44
	I	13	27	43	54	46
	II	13	27	41	53	47
	III	13	28	41	52	48
	IV	14	27	41	54	46

basis. Only 49 of these records, or 7.16 per cent, varied more than 10 per cent from the standard. Reference to table 2 shows that when testing by method II, the increase in variation above 10 per cent from the actual was only 0.58 per cent. In method III, the increase was 5.25 per cent. In method IV, the increase was only 7.31 per cent. In other words, bi-monthly testing was 92.84 per cent dependable; testing three times per lactation was 92.26 per cent depend-

TABLE 5
Accuracy of milk yields for four different types of testing compared to the semi-official test (av. fat-corrected milk)

Breed	S.O. ¹	I	Error	No.	S.O.	II	Error	No.	S.O.	III	Error	No.	S.O.	IV	Error	No.
	(lb.)	(lb.)	(%)		(lb.)	(lb.)	(%)		(lb.)	(lb.)	(%)		(lb.)	(lb.)	(%)	
Ayr. +	11,513	11,671	+1.4	5	10,200	10,436	+2.3	10	10,472	10,973	+4.9	14	11,557	12,060	+4.4	9
Ayr. -	10,168	9,441	-7.1	39	10,357	9,431	-7.2	34	10,249	9,014	-12.0	30	10,003	9,236	-7.7	35
All Ayr.	10,321	9,694	-6.1	44	10,321	9,660	-6.4	44	10,321	9,725	-5.8	44	10,321	9,814	-4.9	44
B.S. +	13,494	13,732	+1.8	16	12,786	13,122	+2.6	25	12,874	13,371	+3.9	34	13,151	13,613	+3.5	22
B.S. -	11,718	11,206	-4.4	73	11,744	11,217	-4.5	64	11,519	10,897	-5.4	55	11,671	10,973	-6.8	67
All B.S.	12,037	11,660	-3.1	89	12,037	11,876	-1.3	89	12,037	11,842	-1.6	89	12,037	11,550	-4.0	89
Guer. +	8,986	9,105	+1.3	12	9,257	9,522	+2.9	27	9,465	9,962	+5.3	41	8,706	8,891	+2.1	11
Guer. -	9,528	9,019	-5.3	76	9,542	9,004	-5.6	61	9,445	8,946	-5.3	47	9,561	8,833	-7.6	77
All Guer.	9,454	9,031	-4.5	88	9,454	9,163	-3.1	88	9,454	9,419	-0.4	88	9,454	8,940	-6.5	88
Jer. +	9,191	9,453	+2.9	16	9,004	9,247	+2.7	28	9,091	9,530	+4.7	33	8,711	9,146	+5.0	15
Jer. -	8,975	8,485	-5.5	66	9,023	8,499	-5.8	54	8,967	8,383	-6.5	49	9,085	8,438	-7.1	67
All Jer.	9,017	8,674	-3.8	82	9,017	8,754	-2.9	82	9,017	8,630	-4.3	82	9,017	8,568	-5.0	82
H.F. +	13,180	13,292	-0.8	82	13,248	13,532	+2.1	124	13,356	13,951	+4.5	180	13,238	13,799	+4.2	100
H.F. -	13,238	12,622	-4.7	299	13,214	12,546	-5.1	257	13,108	12,343	-5.8	201	13,231	12,334	-6.7	281
All H.F.	13,225	12,766	-3.5	381	13,225	12,867	-2.7	381	13,225	13,102	-0.9	381	13,225	12,719	-3.8	381
All +	12,383	12,431	+0.4	131	11,993	12,273	+2.3	214	12,174	12,722	+4.5	302	12,379	12,845	+3.8	157
All -	11,802	11,222	-5.0	553	11,849	11,215	-5.4	470	11,673	10,947	-6.2	382	11,750	10,936	-6.9	527
All	11,894	11,453	-3.7	684	11,894	11,562	-2.8	684	11,894	11,711	-1.5	684	11,894	11,383	-4.3	684

¹ S.O. = semi-official

able; testing the first and last months was 87.59 per cent dependable; and testing the fourth and eighth months was 85.52 per cent dependable.

Table 3 shows the average parameters about the means for fat-corrected milk for all breeds and by individual breeds for the five different methods of testing. The variation for the different methods is slight enough to lack significance. The per cent of variates for all breeds found outside the parameters varies from 11.3 to 12.4 for those above the plus parameters, being 12 per cent for monthly testing; and from 7.4 to 9.9 per cent for those below the minus parameters, being 9.5 per cent for monthly testing. Comparing the four methods of testing directly with semi-official testing, the average plus parameters range from 0.3 per cent to 2.5 per cent lower than those for semi-official testing and the average minus parameters range from 3.9 per cent to 7.8 per cent below. This shows a high degree of dependability for testing less frequently than once a month.

Table 4 shows the per cent of records varying from the mean for the five different methods of testing. According to the results shown here, there is no significant difference between the five different methods of testing insofar as the number of individual records varying from the mean is concerned. In other words, testing once a month or as few times as twice during the lactation makes no difference in the number of records varying from the mean as measured at any particular point. The table shows a check at 1 to 5 per cent, at 1 to 10 per cent, 1 to 15 per cent and at 1 to 20 per cent.

Measured in terms of the means for FCM, as shown in table 5, the average error for all breeds is -3.7 per cent; for method II, the error is -2.8 per cent; for method III, -1.5 per cent; and for method IV, -4.3 per cent.

CONCLUSIONS

Testing every other month is slightly less accurate than testing once a month but is sufficiently accurate for practical application.

Testing three times during the lactation (method III) was only slightly less accurate than testing once a month, there being only 0.58 per cent more variation from the standard than that found in bi-monthly testing.

Testing only twice during the lactation gave sufficiently accurate results, compared to the standard monthly test, to recommend this type of testing under conditions where more frequent tests are difficult to obtain.

Testing three times during the lactation, when the tests are taken on the second, sixth and tenth months, is a sufficiently accurate method to merit a consideration of its adoption as a means toward lowering the cost of testing. Any plan that will increase the number of cows with records will accelerate the improvement of our dairy herds.

REFERENCES

- (1) CANNON, C. Y., FRYE, J. B., AND SIMS, R. Predicting 305-day Yields from Short Time Records. *J. Dairy Sci.*, 25: 991-999. 1942.
- (2) COPELAND, LYNN. Monthly and Bi-monthly Tests. *Jersey Bull.*, 47: 723. 1928.

- (3) GAINES, W. L. Deferred Short-time Test as a Measure of the Performance of Dairy Cows. *J. Agr. Research*, 35: 237-247. 1927.
- (4) GIFFORD, W. The Reliability of Bi-monthly Tests. *J. Dairy Sci.*, 13: 81-90. 1929.
- (5) HOUSTON, J., AND HALE, R. W. The Errors Involved in Certain Methods of Estimating the Lactation Yield of Milk and Butterfat. *J. Dairy Research*, 4, 1: 37-47. 1932.
- (6) M'CANDLISH, A. C., AND M'VICAR, A. Are Milk Record Association Results Accurate? *Scot. J. Agr.*, 8: 201-205. 1925.
- (7) McDOWELL, J. C. Testing Cows for Production Every Other Month. *Holstein-Freisian World*, 24: 1970. 1927.
- (8) McKELLIP, I., AND SEATH, D. W. A Comparison of the Different Methods of Calculating Yearly Milk and Butterfat Records. *J. Dairy Sci.*, 24: 181-192. 1941.
- (9) PETERSEN, W. E. Accuracy of One and Two Day Tests. *Jersey Bull.*, 44: 2044-2059. 1925.
- (10) TURNER, C. W. Seasonal Variations in Milk and Fat Production. *J. Dairy Sci.*, 6: 198-203. 1923.
- (11) TYLER, W. J., AND CHAPMAN, A. B. A Simplified Method of Estimating 305-day Lactation Production. *J. Dairy Sci.*, 27: 463-469. 1944.
- (12) YAPP, W. W. Relative Reliability of Official Tests for Dairy Cows. *Ill. Agr. Expt. Sta. Bull.* 215. 1915.

THE BACTERIAL COUNT, TYRAMINE CONTENT AND QUALITY SCORE OF COMMERCIAL AMERICAN CHEDDAR AND STIRRED CURD CHEESE MADE WITH *STREPTOCOCCUS FAECALIS* STARTER¹

A. C. DAHLBERG AND F. V. KOSIKOWSKY

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

The present investigation was concerned with the use under practical plant conditions of a special strain of *Streptococcus faecalis* that showed promise as a starter for developing flavor in Cheddar and stirred curd cheese (1).

EXPERIMENTAL PROCEDURE

The experimental lots of cheese were made in 3 consecutive days in September, 1947, in a large commercial cheese plant. Three vats of milk were made into cheese on the first day and 4 vats on the next 2 days. One control batch of cheese was made each day with 1.2 per cent of the starter ordinarily used by this cheese company and hereafter referred to as "commercial starter," with no change in the regular procedure used in the plant. As this starter had been developed by one of the large commercial cheese companies, on the second and third days a second control batch of cheese was made using the regular procedure with 1.2 per cent of a good active Hansen lactic starter. The third batch of cheese was made by the regular procedure and the milk was inoculated with 0.8 per cent of Hansen lactic and 1.0 per cent of *S. faecalis* starters. The fourth batch of cheese also was made with 0.8 per cent of Hansen lactic and 1.0 per cent of *S. faecalis* starters, but the stirred curd process was used. All starters were allowed to grow for 1 hour in the pasteurized milk at 86° F. before adding rennet.

The milk was produced in one of the mid-western states on partially dried-out pastures and with some supplementary roughage feeding. Each vat contained 10,000 lb. of milk pasteurized at 162° F. for 70 seconds. The character of the milk was not constant from vat to vat. The fat test varied from 3.5 to 4.0 per cent and the acidity at the time of adding rennet, which was 1 hour after adding the starter, varied from 0.171 to 0.182, expressed as percentage of lactic acid.

The first three batches of cheese were made according to the usual procedure on a definite time schedule of 4 hours and 45 minutes from the addition of rennet to the salting of the curd. The acidity of the whey at the time of salting the curd varied from 0.50 to 0.65 per cent. The fourth batch of cheese was made by the stirred curd process which was identical to the Cheddar process until

Received for publication February 10, 1949.

¹ This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Catherine Werwoert Work for making some of the chemical analyses and to Mr. W. E. Ayres and Mr. J. C. Marquardt, who, with the authors, judged the cheese.

after the whey was drained. The curd was hand and mechanically stirred for 20 minutes. The acidity then was 0.25 or 0.26 per cent and the curd was salted. The total time from the addition of rennet to the salting of the curd was 2 hours and 45 minutes.

During the week of manufacture the cheese was shipped to a cheese storage where it was held at 55 to 60° F. until shipped by refrigerated freight to Ithaca, New York. It arrived within 1 month and then was cured at 50 and at 60° F.

Analyses were made for the usual chemical composition and for tyramine, soluble protein, volatile acids and pH when the cheese was received and after curing for 4 months. The total bacterial count was determined by the standard plate method using tryptone glucose beef extract milk agar and incubating 48 hours at 32° C. The cheese also was plated on sodium azide-penicillin agar and the approximate percentage of enterococci was determined by fishing 10 colonies for identification. This agar contained tryptone, yeast extract, glucose and 0.01 per cent sodium azide to which 100 Oxford units of penicillin per liter were added at the time of pouring the plates. The incubation time was 48 hours at 32° C. The methods have been described previously. The cheese was scored after curing for 2, 4 and 6 months at 50° F., but the scoring was omitted for 6 months at 60° F. as the cheese was overcured and lower in flavor quality. The intensity of the Cheddar flavor was given as mild \pm , medium \pm and sharp \pm . To save space, only the average scores are presented and the intensities were averaged by giving numbers for intensities ranging from 1 to 9.

RESULTS

Sufficient of the manufacturing details were presented under experimental procedure to make it unnecessary to give additional data. The composition of the samples of fresh cheese (table 1) was very uniform, except that the salt content varied more than desired. The moisture contents were sufficiently low to permit good curing for long periods. None of the data on acidity, volatile fatty acids and soluble nitrogen will be presented as they do not assist in evaluating the results.

The standard plate counts of the pasteurized milk in the vats before adding starter varied from 3,000 to 22,000 per ml., and the enterococci-lactobacilli count on azide-penicillin agar varied from 0 to 200 per ml. The bacterial count with azide-penicillin agar on the Hansen starter was known to be 0, whereas the *S. faecalis* starter was 100 per cent enterococci. The commercial starter showed a standard plate count of 760 million per ml. and no growth on azide-penicillin agar.

The first analyses of the cheese for bacteria and tyramine were made 1 month after manufacture (table 2). The Hansen starter cheese showed average total bacterial counts of 34,200,000 per g., yet even in this young cheese the count on azide-penicillin agar was 8,300,000. The cheese showed no enterococci and no tyramine, so one might expect very slow curing cheese (2). However, when this cheese was 4 months old at 50° F. the total counts had increased, and the

TABLE 1

The composition of the experimental Cheddar and stirred curd cheese

Cheese ^a		Moisture	Fat	Salt	Protein	pH
No.	Identity					
		(%)	(%)	(%)	(%)	
919-4	Commercial starter	36.9	33.2	1.63	23.93	5.19
919-5	LDK starters ^b	36.6	32.6	1.18	23.14	5.14
919-6	LDK stirred	36.0	34.2	1.79	24.19	5.32
920-7	Hansen starter	35.6	33.6	1.69	23.84	5.28
920-8	Commercial starter	35.8	34.2	2.12	25.06	5.29
920-6	LDK starters	36.7	33.2	1.50	23.86	5.08
920-5	LDK stirred	35.9	35.8	2.14	23.13	5.23
921-7	Hansen starter	35.6	33.6	1.53	23.96	5.24
921-8	Commercial starter	36.6	32.8	2.09	24.64	5.39
921-6	LDK starters	37.4	32.8	1.41	24.53	5.09
921-5	LDK stirred	35.9	32.8	1.88	23.87	5.18

^a The data for the cheese made with Hansen starter are averages of 2 lots of cheese; all other data are averages of 3 lots of cheese.

^b L starter was Hansen's lactic starter. DK starter was *Streptococcus faecalis*.

azide-penicillin count was 44,800,000 per g., of which 2,200,000 were enterococci. The tyramine content had increased to 102 γ per g. This cheese cured at 60° F. for 4 months gave much lower bacterial counts, but the enterococci count of 3,200,000 was higher. The tyramine content averaged 218 γ per g. so that after curing for 4 months a medium flavor intensity would be expected.

TABLE 2

The average bacterial counts and the tyramine content of Cheddar and stirred curd cheese made on 3 consecutive days

Cheese identity	Bacterial counts (millions omitted)			Tyramine
	Standard agar plate count	Azide-penicillin agar		
		Total count	Enterococci count	
				($\gamma/g.$)
		Cheese 1 mo. old		
Hansen starter ^a	34.2	8.3	0	0
Commercial starter	55.9	15.5	1.1	49
LDK starters	1,373.0	635.0	590.6	65
LDK stirred	785.0	283.3	383.3	99
		Cheese 4 mo. old—last 3 mo. at 45–50° F.		
Hansen starter	53.0	44.8	2.2	102
Commercial starter	63.7	48.7	13.1	290
LDK starters	547.7	475.3	394.5	354
LDK stirred	431.7	183.7	141.4	345
		Cheese 4 mo. old—last 3 mo. at 55–60° F.		
Hansen starter	19.9	6.4	3.2	218
Commercial starter	19.7	1.4	0.3	440
LDK starters	412.7	378.3	367.3	536
LDK stirred	317.7	203.3	183.0	593

^a The data for the cheese made with Hansen starter are averages of 2 lots of cheese; all other data are averages of 3 lots of cheese.

The cheese made with commercial starter gave bacterial counts similar to that made with Hansen starter, except that enterococci always were found in the early stages of ripening and 49 γ of tyramine per g. were present in the cheese when 1 month old. After curing for 4 months, the cheese held at 50° F. contained 290 γ tyramine per g. and at 60° F. the amount had increased to 440 γ . This quantity of tyramine was unexpectedly high for cheese made from pasteurized milk of low bacterial count.

As found previously in cheese made in the laboratory (3), the addition of both Hansen lactic and *S. faecalis* starters produced cheese with very high bacterial counts which survived well during curing. After 1 month the total bacterial content of the cheese was about half *S. faecalis* and this percentage was

TABLE 3

The flavor and body scores of Cheddar and stirred curd cheese made on 3 consecutive days

Cheese identity ^b	Flavor score and rating				Body score	
	Score		Intensity ratings ^a		50° F.	60° F.
	50° F.	60° F.	50° F.	60° F.		
Cheese 2 mo. old						
Hansen starter	40.0	40.3	2.2 mi +	3.4 mi +	29.1	29.3
Commercial starter	39.6	40.0	2.1 mi +	3.3 mi +	28.7	28.9
LDK starters	40.5	40.7	3.2 mi +	3.8 mi +	29.2	29.2
LDK stirred	40.4	40.6	2.8 mi +	3.5 mi +	28.4	28.2
Cheese 4 mo. old						
Hansen starter	40.1	40.2	3.4 mi +	6.7 med +	29.0	29.0
Commercial starter	40.0	39.9	3.7 mi +	6.9 med +	29.0	28.9
LDK starters	41.2	40.9	4.7 med -	7.5 sh -	29.0	28.9
LDK stirred	40.4	40.4	3.5 mi +	6.9 med +	28.2	28.3
Cheese 6 mo. old						
Hansen starter	40.0		5.2 med +		28.6	
Commercial starter	39.0		5.7 med +		29.0	
LDK starters	40.4		6.2 med +		28.9	
LDK stirred	40.7		5.9 med +		28.4	

^a The numbers for flavor intensity are averages based upon numbers given for flavor intensity terms as 1 mild-, 2 mild, 3 mild +, etc., up to 9 sharp +.

^b The data for the cheese made with Hansen starter are averages of 2 lots of cheese; all other data are averages of 3 lots of cheese.

much higher after 4 months. Tyramine production in this cheese was rather rapid, but only slightly more rapid than in cheese made with the commercial starter.

All of the cheese developed flavor rapidly for a product made from pasteurized milk and differences in Cheddar flavor intensities were slight (table 3). However, the regular Cheddar cheese made with both Hansen lactic starter and *S. faecalis* starter (LDK starters) tended to be slightly higher in flavor than the other samples. Both the Cheddar and stirred curd cheese made with lactic and *S. faecalis* starters tended to be highest in flavor scores but the differences were very slight. This slightly higher score for the *S. faecalis* cheese was illustrated by the detailed data not given in table 3. The four judges scored 11

samples of cheese below 40 and none of these samples contained the *S. faecalis* starter. It is interesting to note that the commercial starter produced rapidly-curing cheese of good flavor, but this cheese tended to develop less desirable flavor as aging progressed. This is indicated in the 39 score for the cheese cured 6 months at 50° F. The scores were not taken for all of the cheese ripened at 60° F. for 6 months, and no data are given for this cheese in the table as the cheese was overcured, but the cheese with commercial starter scored 3 points below the *S. faecalis* cheese.

The body of the cheese was scored uniformly, irrespective of the type of starter, but the stirred curd cheese received a lower score due to the open texture.

On several occasions in the laboratory, cheese was made by the stirred curd method from pasteurized milk inoculated with both lactic and *S. faecalis* starters. In all trials, the stirred curd cheese developed normal acidity even though the acid in the whey from the curd was low at the time of salting. *S. faecalis* grew in the salt concentration present in Cheddar and stirred curd cheese and developed acid while the cheese was in the press. In the present plant trials with stirred curd cheese, no time was given for acid development in the curd after drawing the whey. The salt was added to the curd when the whey tested 0.25 per cent acid. The pH of the cheese was normal when taken from the press and the cheese developed normal Cheddar flavor.

DISCUSSION

The commercial Cheddar cheese made by the usual factory workers from pasteurized milk with both lactic and *S. faecalis* starters developed Cheddar flavor of intensity and quality as expected. It was fast-curing cheese of excellent quality. The unexpected part of this study was that the cheese made without *S. faecalis* starter developed both flavor and tyramine almost as rapidly as the cheese to which *S. faecalis* had been added.

It is evident that the pasteurized milk contained small numbers of tyramine-producing bacteria which grew rapidly in the cheese. All samples of cheese gave counts in the millions per gram on azide-penicillin agar when only 1 month old, but some cheese showed no enterococci among the 10 colonies selected per plate. It is assumed that these other bacteria were lactobacilli that grew in the azide-penicillin agar. The cheese made with Hansen starter developed tyramine only as a result of natural inoculation in the milk and the quantities produced were substantial. The bacteria involved may have been solely enterococci, but, if such is the case, relatively small numbers of a few million per gram were ample to produce the tyramine.

The cheese made with commercial starter gave the same approximate bacterial counts as with Hansen starter, but the tyramine content of the cheese was appreciably higher and almost identical to the tyramine content of *S. faecalis* cheese. This problem has not been studied intensively, even though the evidence appears to indicate the presence of tyramine-producing bacteria in the commercial starter. The noteworthy fact is that the cheese to which *S. faecalis*

starter was added contained great numbers of this organism but only slightly increased amounts of tyramine. Hence, it must be concluded that only a few million of *S. faecalis* bacteria per gram of cheese are needed to convert the available tyrosine into tyramine or else other bacteria are able to produce this chemical reaction. That other bacteria may be involved is indicated by the higher tyramine content of cheese made with commercial starter as compared with Hansen starter. Limited trials to isolate from the cheese other bacteria that produced tyramine were unsuccessful, but a direct inoculation in the tyrosine broth with the cheese made without *S. faecalis* starter showed it to produce tyramine rapidly. This may have been due to bacteria other than the enterococci.

It should be evident, therefore, that the addition of *S. faecalis* starter to pasteurized milk will not increase substantially tyramine and flavor production if the natural bacterial population in the milk is ample to produce rapidly curing cheese of excellent flavor. If the cheese tends to cure slowly to relatively flat flavors then *S. faecalis* starter should increase the rate of curing and the quality of the flavor. It seems reasonable to assume that the use of *S. faecalis* starter should give rather uniformly rapid curing with excellent flavors under the widest variety of conditions, but the improvement should be most pronounced in milk of best quality where the infection with tyramine-producing bacteria is the least. The results clearly substantiate the observation that tyramine and flavor production increase together in cheese.

The usual commercial lactic starter may or may not develop sufficient acid in the presence of salt. The ability of *S. faecalis* to develop acid rapidly after salting the curd is very important in several regards. It permits the addition of salt to cheese curd containing insufficient acid with assurance that the correct acidity will develop in the cheese during pressing. This should be an aid in manufacturing Cheddar cheese of more uniform acid content and consequently more uniformly good quality, as slow acid development sometimes has been a cause of poor quality cheese. It also should be a boon to the manufacture of stirred curd. Matting of cheese curd was introduced to permit lactic acid bacteria to produce sufficient acid to control curing properly and to reduce the tendency toward undesirable fermentations, such as gas production. The use of better quality milk properly pasteurized will eliminate undesirable fermentations in the vat and *S. faecalis* starter will assure proper acidity, even after salting. Hence, the time of stirring the curd was reduced to that needed to control moisture, and the making process was reduced by 2 hours and the cured cheese was good typical Cheddar.

CONCLUSIONS

The control cheese made from pasteurized cheese milk with commercial lactic starter contained 290 γ tyramine per g. and gave counts of 13 million enterococci per gram after curing for 4 months at 50° F. The flavor scored 40 and was rated as mild + in intensity. This control cheese cured well.

The addition of *Streptococcus faecalis* starter to the pasteurized milk produced cheese which, after curing for 4 months at 50° F., contained 354 γ tyra-

mine per g. and gave counts of 394 million enterococci per gram. The flavor score was 41.2 and the intensity of flavor was medium-. Similar trends in the data were obtained for cheese ripened at 60° F. Apparently cheese made from pasteurized milk which naturally contained bacteria that ripened the cheese especially well, was improved slightly or not at all by the use of *S. faecalis* starter in the milk.

Cheese of good Cheddar flavor was made by the stirred curd process with *S. faecalis* starter, as much of the desired lactic acid was produced in the cheese after the salting of the curd. The stirred curd method saved 2 hours in the manufacturing process.

REFERENCES

- (1) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Development of Flavor in American Cheddar Cheese Made from Pasteurized Milk with *Streptococcus faecalis* Starter. *J. Dairy Sci.*, 31: 275-284. 1948.
- (2) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Relationship of the Amount of Tyramine and the Numbers of *Streptococcus faecalis* to the Intensity of Flavor in American Cheddar Cheese. *J. Dairy Sci.*, 31: 305-314. 1948.
- (3) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Growth and Survival of *Streptococcus faecalis* in Pasteurized Milk American Cheddar Cheese. *J. Dairy Sci.*, 31: 285-292. 1948.

THE EFFECT OF THE INTERVAL BETWEEN WASHING OF THE UDDER AND ATTACHMENT OF MILKING MACHINES UPON THE MILK PRODUCTION OF DAIRY COWS¹

C. B. KNOTT, J. J. REID, P. S. WILLIAMS AND E. M. KESLER

*Departments of Dairy Husbandry and Bacteriology,
Pennsylvania Agricultural Experiment Station, State College*

During recent years considerable emphasis has been placed upon the importance of rapid milking of dairy cows. This method of milking not only saves time but also is believed to increase milk production and to decrease the incidence of mastitis. It has been observed that many dairymen do not attach milking machines during the recommended period of time after washing a cow's udder. A number of reports (1, 2, 3, 4, 5, 7, 9, 11) has indicated that any delay in removing the milk from a cow following washing of the udder with warm water results in a decrease in milk production.

Data on a series of six trials are presented in this report. These trials were conducted under carefully controlled conditions in an effort to determine the effects of regularly delayed milking, following washing of a cow's udder, upon milk production.

EXPERIMENTAL PROCEDURE

All cows used in these trials were grade Holsteins from the Pennsylvania State College research herds. All cows were milked twice daily. Their udders were washed carefully with water at 125 to 130° F. for approximately 20 seconds. Two or three streams of fore-milk were removed from each teat into a strip cup immediately after washing the udders. Individual interval timers were used for each cow. Machine stripping was followed throughout. All cows were fed and managed similarly and all cows in each trial were housed in the same stable. They were located at random throughout the barn so as to avoid any positional effects. Thus the only known variation was the elapsed time between washing the udders and attachment of milking machines.

In the first trial 30 cows with an average daily milk production of 52 lb. were divided into five groups as nearly alike as possible on the basis of daily milk and milk fat production, stage of gestation and stage of lactation. Milking machines were attached 2, 4, 6, 8 and 10 minutes after the washing was started. This trial was conducted for 35 days at each of the two milkings per day.

It was necessary, because of the need for cows for other experiments on pasture management, to discontinue 15 of the cows on the first trial after 35 days. Accordingly, 15 cows remaining in the 2-, 6- and 10-minute groups of the first trial were continued for an additional 35 days. The data covering the 70 days

Received for publication March 4, 1949.

¹ Authorized for publication as paper no. 1508 in the Journal series of the Pennsylvania Agricultural Experiment Station.

with the latter group of 15 cows is referred to as the second trial. The cows used in the second trial were carried for 70 days with elapsed times of 2, 6 and 10 minutes between washing and attachment of machines at each milking.

In the third trial, 16 cows with an average daily production of 40 lb. per day were divided into four similar groups. In this trial the time intervals were 2, 5, 10 and 20 minutes and the trial was conducted for 90 days.

In the fourth trial, 12 cows with an average daily milk production of 45 lb. were divided into four similar groups. In this trial, the cows all were fed on the basis of 110 per cent of the Morrison (8) standards for good cows under usual conditions. The hay and silage fed were identical in amount for all cows on the trial. The daily grain ration was changed every 2 weeks on the basis of milk and milk fat production for 5 days previous and also on the basis of body weight at the beginning of each 2-week period. This trial was conducted for a period of 84 days.

TABLE 1

The effect of delayed milking following washing of cows' udders upon milk production (first trial)^a

Days	Milk production with time intervals of:				
	2 min.	4 min.	6 min.	8 min.	10 min.
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
- 5 thru - 1	50.4	56.3	50.8	51.6	52.7
1 thru 5	49.8	55.2	50.5	49.4	51.0
31 thru 35	49.2	52.4	49.3	46.9	50.8
Decrease	1.2	3.9	1.5	4.7	1.9

^a Six cows were used per group. Data are expressed in terms of mean daily milk production.

In the fifth and sixth trials, experiments were designed to compare the production of halves of udders, in which whole udders were delayed 12 minutes but each half was milked simultaneously in a quarter-section milker,² to production of halves of udders of cows milked 2 and 12 minutes, respectively, following the beginning of washing. These two trials were designed to explain the discrepancies between previous results published by Knott *et al.* (6) and those of Ward and Smith (11).

Analyses of variance were conducted according to the methods of Snedecor (10) wherever applicable.

RESULTS

A summary of the data obtained in the first trial will be found in table 1. Where recommended procedure was followed, as in the 2-minute group, there was a mean daily decline of 1.2 lb. of milk during the 35-day period. In those groups in which there was a delay of 4, 6, 8 or 10 minutes prior to the attachment of machines following the beginning of washing, the respective mean declines were 3.9, 1.5, 4.7 and 1.9 lb. of milk per cow per day. There were no

² The authors wish to express their appreciation to the De Laval Separator Co., New York, N. Y., for the equipment used in these trials.

observable immediate declines or variations in milk production at the beginning of or during this trial. An analysis of variance proved there was no statistically significant effect of treatment in this trial as far as milk production was concerned.

Fifteen cows from the first trial were continued for an additional 35 days. During the first trial, these cows were in the 2-, 6- and 10-minute groups and were continued for an additional 35 days, making a total of 70 days in what is referred to as the second trial (table 2). These three groups were comparable

TABLE 2
The effect of delayed milking following washing of cows' udders upon milk production (second trial)^a

Days	Milk production with time intervals of:		
	2 min.	6 min.	10 min.
	(lb.)	(lb.)	(lb.)
- 5 thru -1	54.7	52.2	55.5
1 thru 5	54.3	50.8	54.0
66 thru 70	41.8	41.4	45.6
Decrease	12.9	10.8	9.9

^a Five cows were used per treatment. Data are expressed in terms of mean daily milk production.

in milk production, stage of lactation and stage of gestation. During the course of this trial there was a decline of 12.9, 10.8 and 9.9 lb. of milk per cow per day for the 2-, 6- and 10-minute groups, respectively. An analysis of variance proved that the differences in milk production shown in table 2 were not significant.

TABLE 3
The effect of delayed milking following washing of cows' udders upon milk production (third trial)^a

Days	Milk production with time intervals of:			
	2 min.	5 min.	10 min.	20 min.
	(lb.)	(lb.)	(lb.)	(lb.)
- 5 thru -7				
0	40.2	37.0	41.1	40.8
1 thru 10	40.4	37.7	41.4	39.1
81 thru 90	36.8	29.4	31.7	29.2
Decrease	3.4	7.6	9.4	11.6

^a Four cows were used per treatment. Data are expressed in terms of mean daily milk production.

The data for the third trial are presented in table 3. The time intervals between the beginning of the washing of the cows' udders and the attachment of the machines were 2, 5, 10 and 20 minutes, respectively. During the 90-day period during which this trial was conducted, declines of 3.4, 7.6, 9.4 and 11.6 lb. per cow per day were found for the 2-, 5-, 10- and 20-minute groups, respectively. While the differences were quite large between the mean declines in

daily milk production of the various groups, actually these differences were not statistically significant. There was considerable variation in the response of various cows. One cow in the 5-minute group declined 17.7 lb. per day and two cows in the 10-minute group declined 20.8 and 16.3 lb. per day, respectively, but two others decreased only 1.4 and 1.1 lb. per day, each, during the 90 days of this trial. Apparently these decreases were not the result of the delay in milking but, rather, were due to a lack of persistency of the cows involved.

As a result of the variable responses observed in the third trial as compared to the other two, a fourth trial was conducted. These data are presented in table 4. In this experiment, as in trial 3, two cows that were lacking in per-

TABLE 4

*The effect of delayed milking following washing of cows' udders upon milk production (fourth trial)**

Days	Milk production with time intervals of:			
	2 min.	5 min.	10 min.	20 min.
	(lb.)	(lb.)	(lb.)	(lb.)
- 7 thru - 1	44.4	44.3	45.4	45.1
1 thru 7	42.9	43.4	39.5	42.9
78 thru 84	22.8	37.9	42.3	42.8
Decrease	21.6	6.4	3.1	2.3

* Three cows were used per treatment. Data are expressed in terms of mean daily milk production.

sistency unavoidably were placed in the 2-minute group. One of these declined 22.5 and the other 38.0 lb. per day during the course of this trial. Production of the other cow in the 2-minute group declined only 0.5 lb. per day during the 84-day experimental period. In those groups in which milking was delayed 5, 10 and 20 minutes there were mean daily decreases of 6.4, 3.1 and 2.3 lb. of milk respectively. The decreases in production of the latter three groups are considered equal to or less than the normal decline in production to be expected in such a period of time.

All cows in trials 5 and 6 were milked for 5 days with a delay of 2 minutes between the beginning of washing of the udders and attachment of the machines. The production of each half was determined on each of the twice-daily milkings. It will be observed in table 5 that in those cows in which one-half of the udder was delayed 2 minutes and the other half 12 minutes that the mean daily milk production per half declined 0.1 and 3.3 lb., respectively. However, when milking was delayed 12 minutes on both halves, the mean daily declines were only 0.6 and 0.3 lb., respectively.

While a reversal trial is open to question because of the apparent carry-over effects of previous treatment, the same groups of cows immediately were milked for a period of 5 days involving a 2-minute delay as presented in trial 6. The left halves of the udders of the cows of group A did not recover in milk production during the 5-day control period at the beginning of trial 6 (table 6).

TABLE 5

The effect of delayed milking following washing of cows' udders upon milk production (fifth trial)^a

Group	A		B	
Half of udder	R. H.	L. H.	R. H.	L. H.
Delay (min.)	2	2	2	2
Days	(lb.)	(lb.)	(lb.)	(lb.)
1	27.0	27.6	26.1	28.5
2	27.2	27.9	26.4	28.4
3	27.7	27.7	26.5	28.8
4	28.3	28.4	27.1	28.1
5	27.9	29.3	27.1	28.0
Av.	27.6	28.0	26.6	28.3
Delay (min.)	2	12	12	12
1	27.5	23.2	26.7	28.0
2	27.8	20.6	26.0	29.2
3	27.9	19.7	25.9	28.5
4	27.1	20.0	25.9	27.5
5	27.4	20.0	25.8	27.0
Av.	27.5	24.7	26.0	28.0
Decrease	0.1	3.3	0.6	0.3

^a Each datum represents mean total daily milk production per half-udder for four cows.

TABLE 6

The effect of delayed milking following washing of cows' udders upon milk production (sixth trial)^a

Group	A		B	
Half of udder	R. H.	L. H.	R. H.	L. H.
Delay (min.)	2	2	2	2
Days	(lb.)	(lb.)	(lb.)	(lb.)
1	28.2	26.2	25.8	27.2
2	28.9	26.0	26.4	28.0
3	28.7	26.1	26.5	28.4
4	28.6	26.4	26.7	27.9
5	28.1	26.8	27.1	28.0
Av.	28.5	26.3	26.5	27.9
Delay (min.)	12	12	2	12
1	27.3	26.8	25.8	26.8
2	26.4	25.9	26.4	26.8
3	27.0	25.4	26.5	25.5
4	26.9	25.9	26.7	25.4
5	28.9	27.5	27.1	25.9
Av.	27.3	26.3	25.3	26.0
Decrease	1.2	0.0	1.2	1.9

^a Each datum represents mean total daily milk production per half-udder for four cows.

Following this period, the groups were reversed and these data are presented in table 6. The greatest decline in mean daily milk production also occurred in those halves delayed in milking for 12 minutes of cows in which the other half was delayed 2 minutes in milking following preparation. In trial 6 in those cows where both groups of halves of the udders were delayed 12 minutes prior to milking, the change in mean daily milk production was 0.0 lb. and only equal to that of those half-udders delayed for 2 minutes on the other group of cows in this trial, respectively. Analysis of variance of these data indicates that the declines in production were not significant where entire udders were delayed for 12 minutes. However, the declines in mean daily milk production were highly significant when delays of 12 minutes were incurred and compared to the other halves of the udders that had been milked after a 2-minute delay. This raises a question relative to attempting to draw conclusions from half-udders or from quarters in experiments of this type.

In only exceptional cases did the cows fail to let down their milk to the stimulation of preparation. The incidence of this was not related to treatment.

DISCUSSION

The series of six trials reported was conducted in an effort to obtain additional data emphasizing the importance of rapid milking following the washing of cows' udders. These data indicate that an interval of up to 20 minutes between the beginning of washing of a cow's udder and attachment of milking machines does not affect milk production. Apparently the cows were able to condition themselves immediately to a change in procedure from a 1- to 2-minute routine prior to these trials.

These data are in disagreement with the results obtained by Miller and Petersen (7) and Ward and Smith (11). The mean levels of production of the cows studied by the above authors were considerably below those reported in this publication, indicating that they may have been nearer to the end of the lactation period. In addition, the work of Ward and Smith (11) may have been confounded by the effects of milking one half of the udder upon production of the delayed half as indicated by the data presented in this report. Further, their study was conducted upon only five cows as compared to 66 used in the trials in these experiments. The variations between cows are extremely large and also may have been a factor.

The data presented show no marked declines in milk production during the first week to 10 days in any of the groups of cows used in the first four trials. Conclusions from these data, however, should not affect in any way the routine followed in good milking procedures. A method should be followed in which the cows' udders are washed 1 to 2 minutes prior to the attachment of machines because of efficiency of labor and production of clean milk. Occasional cows may lie down in their stalls if delays of longer than 2 minutes are incurred following washing. Their udders then will have to be washed again to produce clean milk.

SUMMARY

Regular delays up to 20 minutes from the beginning of washing cows' udders until the attachment of milking machines apparently did not decrease milk production under the conditions of these trials. Routine fast milking procedures, however, should emphasize the importance of attaching machines 1 to 2 minutes after washing because of labor efficiency and production of clean milk. Data are presented which emphasize the importance of using entire udders in drawing conclusions in work of this type.

REFERENCES

- (1) BABCOCK, S. M. Variations in the Yield and Quality of Milk. 6th Annual Rept., Wis. Agr. Expt. Sta. Pp. 42-62. 1889.
- (2) BEACH, C. L. The Milk Yield of Quarters on the Same Side of the Udder. Conn. Agr. Expt. Sta. Rept. Pp. 135-137. 1904.
- (3) BITTING, A. W. The Physiology of Milk Secretion. 14th Annual Rept. Indiana Agr. Expt. Sta. Pp. 51-88. 1901.
- (4) DODD, J. H., AND FOOT, A. S. Experiments on Milking Technique. 1. Effect of Washing the Udder with Hot Water. 2. Effect of Reducing Milking Time. J. Dairy Research, 15: 1-17. 1947.
- (5) EMERY, F. E. Milk Records and Tests. North Carolina Agr. Expt. Sta. Bull. 116. 1895.
- (6) KNOTT, C. B., REID, J. J., WILLIAMS, P. S., AND KESLER, E. M. The Effect of the Interval Between Washing of the Udder and Attachment of Milking Machines Upon the Bacterial Flora and Milk Production of Dairy Cows. J. Dairy Sci., 30: 580-581. 1947.
- (7) MILLER, K., AND PETERSEN, W. E. Some Factors Involved in Efficient Milking. J. Dairy Sci., 24: 225-233. 1941.
- (8) MORRISON, F. B. Feeds and Feeding. The Morrison Pub. Co., Ithaca, N. Y. 1936.
- (9) SKRODEL, L. Der Einfluss der Milchgeschwindigkeit auf die Sekretion der Milch und des MilCHFettes. Milchw. Forsch., 19: 95-105. 1937.
- (10) SNEDECOR, G. W. Statistical Methods. Iowa State College Press, Ames. 1946.
- (11) WARD, G. M., AND SMITH, V. R. Total Milk Production as Affected by Time of Milking after Application of a Conditioned Stimulus. J. Dairy Sci., 32: 17-21. 1949.

THE EFFECT OF THE ASCORBIC ACID CONTENT OF FLUID MILK UPON THE KEEPING QUALITY OF ITS DRIED PRODUCT

PHILIP A. WRIGHT AND GEORGE R. GREENBANK

*Bureau of Dairy Industry, Agricultural Research Administration,
U. S. Department of Agriculture*

Milk freshly drawn from the udder contains an average of 22 to 23 mg. per l. of ascorbic acid, present entirely in the reduced form, according to Knight *et al.* (4) and Kon and Watson (5, 6). As soon as milk is drawn, however, it is exposed in a greater or less degree to the oxygen of the air, to light and perhaps to contaminating metals. These all serve, according to Kon and Watson (6) and Whitnah *et al.* (7), to reduce the amount of ascorbic acid until, after a 24-hour period, only a small fraction of the original amount may be left.

Since ascorbic acid is oxidized rather easily, it is possible that it plays an important role as a retarder of oxidative deterioration. While considerable work has been reported on the effect of ascorbic acid added to fluid milk, reports of the effect of ascorbic acid added to fluid milk on the keeping quality of its dried product are conflicting (1, 2); therefore, it was decided to add ascorbic acid in dilute water solution to fluid milk and determine the effect of the addition on the keeping quality of the dried product.

EXPERIMENTAL PROCEDURE

All fluid milks used were mixed samples from the same herd and, while considered to be of average to good quality, were not selected milks. Four groups of dried whole milk were prepared from different herd milks. In preparing each group a large quantity of herd milk was standardized at 3.3 per cent fat and divided into four equal lots. One lot was used as a control and was concentrated and spray-dried without the addition of ascorbic acid. Increasing amounts of ascorbic acid in dilute aqueous solution were added to the remaining lots. To the second, third and fourth lots, 5, 10 and 20 mg. per l., respectively, were added. These additions were equivalent to increases of approximately 25, 50 and 100 per cent, respectively, based on a normal estimated ascorbic acid concentration of 20 mg. per l. in fresh raw milk several hours old. All four lots were preheated to 170° F. for 30 minutes, concentrated in a vacuum pan and spray-dried. The dry milk was collected in 10-gallon milk cans which were closed tightly and held overnight at 34° F. The following morning the cans and contents were allowed to come to room temperature and the milk was weighed into small tin cans holding approximately 70 g. each. The cans were punctured and evacuated under 2 mm. pressure for 1 hour, the vacuum released with nitrogen and the holes soldered. The cans of dry milk were placed in storage at 98.6° F. Sample cans were removed at 2-week intervals and the milk reconstituted and judged for flavor. Moisture in the dry milks of group

Received for publication March 7, 1949.

1 ranged from 2.5 to 3 per cent, of groups 2 and 4, from 1.6 to 1.8 per cent and of group 3, 1.3 per cent. Fat content was approximately 26.7 percent in all samples.

RESULTS

Table 1 shows the apparent ascorbic acid content (all those substances which reduce 2,6, dichlorophenolindophenol) of the fluid milk in the control sample

TABLE 1

The apparent ascorbic acid content of the control samples of raw milk and their dried products as soon as prepared

Group no.	Raw milk	Reconstituted dried milk
	(mg./l.)	(mg./l.)
1	15.6	15.1
2	15.6	10.4
3	15.0	4.6
4	14.8	4.2

of each group and the concentration in the freshly prepared dried whole milk when reconstituted. While there is no appreciable variation in the concentration of apparent ascorbic acid in the fluid milk of each group, there is a marked difference in the concentration of apparent ascorbic acid in the reconstituted dried milk. Milks seem to vary in the degree of retention of apparent ascorbic acid content during processing.

A comparison of the data in tables 1 and 2 shows that those samples which retained the greatest amount of apparent ascorbic acid during processing have the best keeping quality. To what extent this destructive action accompanying heating can be used as a criterion of the keeping quality of the dried product is being determined.

TABLE 2

The effect of increasing the ascorbic acid content of fluid milk before heat treatment on the keeping quality of its dried product

Ascorbic acid added	Keeping quality of dried product at 37° C.			
	Group 1	Group 2	Group 3	Group 4
(mg./l.)	(d.)	(d.)	(d.)	(d.)
0	364	213	38	70
5	405	395	68	140
10	419	443	144	182
20	600	456	181	295

The data in table 2 are based on the time required for the stored milk to become so tallowy as to be inedible. These data indicate that ascorbic acid added to the fluid milk before preheating increases the keeping quality of its dried product. In every series of dry milks, the larger the amount added, the better the keeping quality. However, no recommendation can be made on the

basis of these experiments as to just how much ascorbic acid should be added to keep the dried milk edible for a given number of days.

The keeping quality of the dried milk in group 1 to which 20 mg. per l. of ascorbic acid were added was exceptional. This dried milk sample never did become tallowy. It finally was judged inedible because of other flavors that developed from long storage at 37° C. The concentration of apparent ascorbic acid of this milk never dropped below 16 mg. per l. and after storage for 20 months was greater than immediately after drying.

Figure 1 shows the change in the apparent ascorbic acid of this milk and also of a milk with poor keeping quality with no added ascorbic acid. A study of

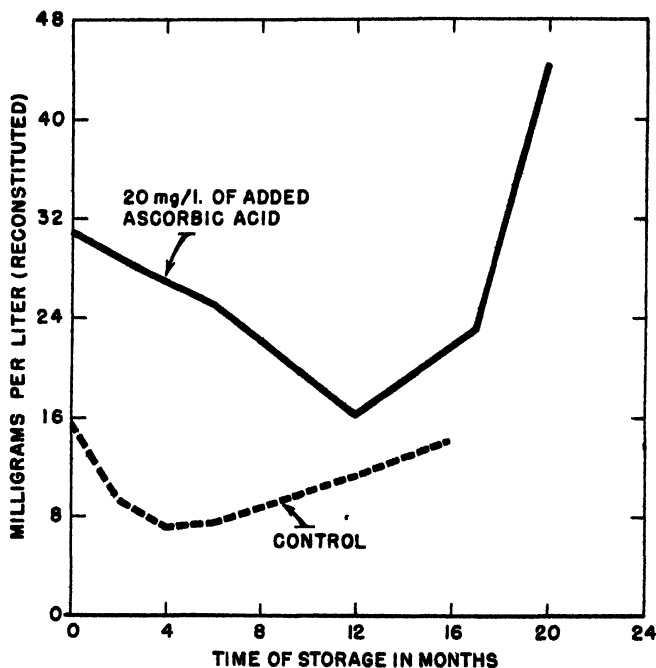


FIG. 1. The change in apparent ascorbic acid during storage at 37° C. (98.6° F.)

this figure indicates that reducing substances are formed in storage and that in a dried milk with good keeping quality the apparent ascorbic acid, while dropping to a certain minimum value, seems afterwards to increase until there appears to be more apparent ascorbic acid present than in the dried milk freshly made. On the other hand, in the milk marked "control," the concentration of apparent ascorbic acid drops to a lower value than does that of the milk of good keeping quality, which apparently allows the oxidation to proceed and produce tallowiness and other off flavors.

The results in figure 1 illustrate the changes in reducing capacity in two dried milks which differ greatly in their keeping quality. It would seem that

to retard oxidation materially a dried milk must have an initial reducing capacity of such magnitude that even after a loss in this characteristic a sufficient reducing action remains to prevent the onset of oxidation until the product can form its own reducing substances. If a product does not possess a high reducing capacity initially, the loss in this property makes possible the early onset of oxidative reactions and results in a product of low keeping quality.

Since it had been suggested by Guthrie (3) that the complete destruction of ascorbic acid in milk prior to its pasteurization might inhibit the development of the tallowy flavor, several lots of dry milk from fluid milk in which the ascorbic acid had been oxidized with hydrogen peroxide were prepared to find out what effect the complete absence of reduced ascorbic acid would have on the keeping quality of the dry milk. Three pairs of dry milks were prepared, using the same heat treatment and storage temperatures as with those described in table 1. To one fluid milk of each pair just enough dilute hydrogen peroxide was added carefully to oxidize the ascorbic acid completely. The results shown in table 3 indicate that the dried products from the milks to which hydrogen peroxide was added became tallowy more rapidly than those where no hydrogen peroxide was added.

TABLE 3

The effect of oxidizing the ascorbic acid in the fluid milk with hydrogen peroxide on the keeping quality of its dried product

Pair no.	Time to become inedible at 37° C. (98.6° F.)	
	Normal ascorbic acid	Ascorbic acid oxidized by H ₂ O ₂
	(d.)	(d.)
1	308	239
2	231	189
3	266	224

CONCLUSIONS

1. Ascorbic acid added to milk before drying increases the keeping quality of its dried product.
2. Oxidation of ascorbic acid in the fluid milk with hydrogen peroxide does not increase the keeping quality of its dried product.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Mr. C. F. Hufnagel of these laboratories for his work in preparing the dry milks used in the experiments reported in this paper.

REFERENCES

- (1) COULTER, S. T., AND JENNESS, R. The Relation of Ascorbic Acid to the Keeping Quality of Dry Whole Milk. (Abs.) J. Dairy Sci., 30: 528. 1948.
- (2) GRAY, P. P., AND STONE, I. New Method of Preventing Rancidity. Food Industries, 11: 626-628. 1939.
- (3) GUTHRIE, E. S. Quality Control in Dairy Plants. Am. Milk Rev., 9: 18-26. 1947.

- (4) KNIGHT, C. A., DUTCHEE, R. A., AND GUERRANT, N. B. The Quantitative Determination of Vitamin C in Milk. *Science*, **89**: 183-185. 1939.
- (5) KON, S. K., AND WATSON, M. B. The Vitamin C Content of Cows' Milk. *Biochem. J.*, **31**: 223-226. 1937.
- (6) KON, S. K., AND WATSON, M. B. The Effect of Commercial Pasteurization on the Vitamin C in Milk. The National Institute for Research in Dairying and the Rowett Research Institute. Unnumbered publication. Part 1: 52-56. 1937.
- (7) WHITNAH, C. H., RIDDELL, W. H., AND CAULFIELD, W. J. The Influence of Storage, Pasteurization and Contamination with Metals on the Stability of Vitamin C in Milk. *J. Dairy Sci.*, **19**: 373-383. 1936.

THE EFFECT OF A PERIOD OF NON-MILKING ON THE LEUCOCYTE COUNT OF MILK

E. O. ANDERSON AND PATRICIA MACLEOD

*Department of Animal Industry, Storrs Agricultural Experiment Station,
University of Connecticut, Storrs*

This paper reports an experiment on the effect of interrupted milking upon the leucocyte count of milk. A parallel study on the effect of interrupted milking upon milk production and the vitamin A and carotene content of milk will be reported elsewhere.

METHODS

Cows in the middle of their lactation periods and free from mastitis (2) were used, six as experimental animals and two as controls. During the experiment they were on a controlled ration and, except as noted below, were milked at 12-hour intervals. Representative samples of milk were taken from each cow at each milking for 3 days. Then for 10 days the six experimental cows were not milked but the two control animals were milked as usual. After 10 days, normal milking schedules were resumed on the experimental cows and milk samples were collected at each milking for 3 days. The milk samples from the first 3 days are referred to as the "pre" samples and those from the last 3 days as the "post" samples.

Milk films were prepared from each sample, defatted in xylol and stained with Newman-Lampert stain (1). Sixty microscopic fields were examined on all "pre" milk films and the average number of leucocytes determined as described in "Standard Methods" (1). The "post" milk films were treated similarly except that in most cases fewer microscopic fields were examined because of the higher level of leucocytes.

For statistical analysis the leucocyte counts were converted to logarithms. The difference between the logarithms of the "pre" and "post" counts of the milk from the experimental animals was compared with the corresponding difference from the milk of the controls by an analysis of variance.

RESULTS AND DISCUSSION

The leucocyte count of the milk from the control cows varied little throughout the entire experiment but that from the experimental animals increased significantly after the period of non-milking. The geometric mean leucocyte count of the "pre" samples of the experimental animals was approximately 100,000 leucocytes per ml., and that of the "post" samples 4,860,000 leucocytes per ml. The individual counts and records of milk production at each milking are given in table 1. The corresponding leucocyte counts of the controls averaged 180,000 leucocytes per ml. during the "pre" period and 300,000 leucocytes per ml. during the "post" period, which may be compared with the average count of the control animals for the entire 16 days of 200,000 leucocytes per ml.

Received for publication March 16, 1949.

TABLE 1
Leucocyte counts on "pre and post interruption" milk samples and milk production records of six experimental cows and two control cows

Cow	Pre sample				Post sample			
	1st d.		2nd d.		1st d.		2nd d.	
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
	Leucocyte counts (millions/ml. of milk)							
A	0.13	0.16	0.11	0.17	0.96	0.95	4.98	4.86
B	0.14	0.01	< 0.01	0.01	0.05	0.08	8.40	5.64
C	0.01	0.04	0.02	0.04	0.02	0.10	9.72	8.40
D	0.73	0.89	0.21	0.46	0.83	0.26	11.34	6.72
E	0.06	0.07	a	0.32	0.15	0.21	3.60	2.52
F	0.15	0.07	0.07	0.13	0.06	0.08	15.00	17.10
Control G	0.27	0.31	0.26	0.28	0.22	0.33	0.49	0.48
Control H	0.09	0.08	0.05	0.30	0.30	0.04	0.10	0.08
	Milk production (lb.)							
A	7.7	6.1	8.2	5.7	7.3	6.2	1.7	0.5
B	12.6	10.6	13.0	10.1	12.6	9.5	2.6	0.6
C	11.2	8.9	11.3	8.2	10.4	7.7	2.4	0.6
D	9.5	8.1	9.6	7.8	8.5	7.6	0.9	0.5
E	9.2	8.6	10.7	8.3	10.8	1.9	3.9	8.8
F	8.9	7.0	8.9	7.1	8.2	7.0	1.4	0.5
Control G	13.6	11.6	14.7	10.7	13.6	10.5	14.3	10.9
Control H	9.6	9.1	9.3	8.8	9.3	7.6	9.5	6.1
							10.4	9.2
							12.6	6.8
							10.9	9.3
							1.2	1.2
							1.3	1.3
							1.1	1.1
							0.6	0.9
							2.3	3.1
							0.7	0.7
							14.3	10.9
							9.2	9.3

a No sample.

The higher leucocyte counts of the "post" samples as compared with the "pre" samples is evident from table 1. No marked difference occurred in the leucocyte counts of the milk from the control cows. An analysis of variance of the differences between the "pre" and "post" log counts showed that the increased count in the experimental animals was significantly greater ($P < 0.02$) than the corresponding difference in the samples from the control cows.

Since the only known variable between the experimental and control cows was the milking schedule, the increased leucocyte counts of the "post" samples from the experimental animals may be attributed to the 10-day interruption in milking. The ratio in logarithms of the relative increase in milk from the experimental cows to that from the control cows was 1.439 ± 0.433 . In this experiment non-milking decreased milk production 85 per cent and increased the leucocyte count about 27-fold. The confidence limits of the true proportional increase in leucocyte count varied from a factor of 2.4 to a factor of 316 at odds of 19 in 20.

SUMMARY

Interruption in the milking of normal cows in the middle of their lactation cycles increased the leucocyte count of the milk about 27-fold.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. C. I. Bliss, Biometrician, Storrs Agricultural Experiment Station, for his help in the statistical analysis of the data, and to Dr. H. D. Eaton, Dept. of Animal Industry, University of Connecticut, for the data on milk production.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 8th ed. New York, N.Y. 1941.
- (2) PLASTRIDGE, W. N., ANDERSON, E. O., WILLIAMS, L. F., AND HALE, H. H. Infectious Bovine Mastitis. 9. Information on the control of Chronic Mastitis. Storrs Agr. Expt. Sta. Bull., 255. 1946.

THE EFFECT OF *STREPTOCOCCUS LACTIS* AND COLIFORM ORGANISMS ON THE SOLUBLE NITROGEN OF MILK

E. B. COLLINS AND F. E. NELSON

Dairy Industry Department, Iowa Agricultural Experiment Station, Ames

The results of various investigators have shown that many strains of *Streptococcus lactis* are capable of bringing about protein degradation in milk. Early investigations concerning proteolysis of *S. lactis* as measured by soluble nitrogen, by amino nitrogen (determined by the Van Slyke procedure, formol titration or the nitrogen fraction soluble in phosphotungstic acid) and by ammonia determinations, were reviewed by Hammer and Patil (4). In most of these early investigations trials were carried out after periods of incubation ranging from 1 week to 4 months, using milk to which amounts of calcium carbonate sufficient to neutralize all, or nearly all, of the acid that could be produced had been added. Hammer and Patil (4) incubated several strains of *S. lactis* in milk and determined nitrogen soluble in acetic acid and Van Slyke amino nitrogen after periods of incubation ranging from that required for coagulation to 9 days. They demonstrated that a definite proteolysis is evident in many freshly coagulated cultures.

Very little work has been published on proteolysis by *Escherichia coli* and *Aerobacter aerogenes*. Kendall *et al.* (5) reported that *E. coli* had little action upon the protein constituents of milk, as shown by measurements of ammonia content. Spitzer *et al.* (8) found that at 15 days *E. coli* had produced no ammonia but had produced detectable amino acids and peptones, while *A. aerogenes* had produced detectable ammonia and no detectable amino acids or peptones. They used Folin's aeration method for ammonia and precipitation with zinc sulfate and phosphotungstic acid for peptone and amino acid determinations. Berger *et al.* (2) demonstrated the production of peptidases by *E. coli* growing in a peptone glucose medium. Staffe (9) found that growth of *E. coli* in milk at 30° C. causes an increase in the nitrogen soluble in methyl alcohol. These increases were detectible at 1 week, and the nitrogen soluble in methyl alcohol continued to increase throughout the 7-week experimental period. Taylor (10) grew *E. coli communis* in milk for 5 months, after which he coagulated the casein with acid and filtered the mixture. Because of the large amount of precipitate obtained from the whey when phosphotungstic acid was added and the negative Biuret test on the filtrate, he concluded that casein was digested mainly to proteoses and peptones with the formation of only a small percentage of amino acids.

EXPERIMENTAL

In this work proteolysis by *S. lactis*, *E. coli* and *A. aerogenes* growing separately and in combination at 30° C. in skim milk in the absence of calcium car-

Received for publication March 25, 1949.

Journal paper no. J-1627 of the Iowa Agricultural Experiment Station, Ames. Project no. 1023.

bonate for periods up to 15 days was studied. Nitrogen soluble and insoluble in trichloroacetic acid, total nitrogen, titratable acidity, plate count and differential count (where two or more organisms were concerned) were determined.

All organisms used were isolated from samples of raw milk and raw cream just prior to the beginning of the study. Only active strains were used. All strains of *S. lactis* were typical members of the species as characterized by Sherman (7). *E. coli* and *A. aerogenes* were typical according to *Bergey's Manual of Determinative Bacteriology* (3). The stock cultures were transferred once a week, using sterile litmus milk. Cultures were transferred every 24 hours for two or three transfers prior to each trial. One-tenth of 1 per cent inoculum was used in all cases. For trials in which two or more different organisms were used, cultures 20 to 24 hours old were mixed in equal quantities; after the mixture had been shaken thoroughly, the desired inoculum was withdrawn and added to the test bottles of milk. Cultures were incubated at 30° C. to obtain a high rate of change in the substratum without inhibiting the growth of one or more types of organisms used. Data on uninoculated control samples are not included since spore-forming organisms caused rapid decomposition of such samples; however, the presence of acid-producing bacteria prevented the growth of significant numbers of spore-forming organisms. This was evidenced by the absence of any significant number of colonies of spore-forming organisms on the plates incubated for total bacterial count and the absence of abnormal fermentation in the inoculated cultures.

Milk for the trials consisted of fresh skim milk which was heated at 185° F. for 20 minutes to destroy as many undesirable organism types as possible without subjecting the milk constituents to the changes that would accompany sterilization in the autoclave. Skim milk was chosen because the absence of fat facilitated accurate sampling and analysis. Four-ounce screw top bottles were used as containers, and a separate bottle containing 100 ml. of milk was used for each day of testing. At the end of the incubation period the loss in weight, due to evaporation, was made up by adding sterile distilled water to each bottle. With trials using only *S. lactis*, caps were screwed down tightly; in trials where *E. coli* and *A. aerogenes* were involved, caps were left loose to permit the escape of gases.

Coagulated samples were blended for 5 minutes in a sterile Waring blender with a semi-micro attachment and then transferred to a 250-ml. Erlenmeyer flask. Foam was dispersed by adding 8 to 10 drops of n-octyl alcohol to each sample in which coliform organisms had grown. This latter practice was not employed in trials with *S. lactis*, and foam undoubtedly was responsible for the occasional variation in total nitrogen values. Extreme care was used to agitate the undiluted and the diluted samples thoroughly just prior to pipetting, since there was some tendency for precipitation in coagulated samples, even after they had been blended for 5 minutes. Blending for longer periods did not overcome this tendency. A 50-g. quantity of blended sample was weighed into a 100 ml. volumetric flask and the remainder of the volume was made up with

distilled water. A 10 ml. quantity of this solution, representing 5 g. of sample, was used for each nitrogen determination. Nitrogen fractions insoluble in trichloroacetic acid were precipitated by the method developed for cheese serum by Lane and Hammer (6), except that in this work 10 ml. portions of the diluted sample were used, while Lane and Hammer (6) used 1 ml. of cheese serum. All nitrogen determinations were made by the Kjeldahl procedure. Duplicate determinations were made in all cases.

Samples for total bacterial counts and/or differential count were taken directly from the blender in case of coagulated samples and from the sample bottle when blending was unnecessary because the culture had not coagulated. Total counts were made on standard milk agar and differential counts were made on violet red bile agar, according to *Standard Methods for the Examination of Dairy Products* (1), except that a temperature of 30° C. was used for incubation of plates for total counts.

RESULTS

Determinations were run on four strains of *S. lactis*; one strain of *E. coli*; one strain of *A. aerogenes*; and a combination of two strains of *S. lactis*, *E. coli* and *A. aerogenes*.

TABLE 1

Proteolysis by Streptococcus lactis in heated skim milk (av. of duplicate determinations)

Culture	Age	Titrateable acidity	Plate count	Sol. N
	(d.)	(%)	(/ml.)	(% of total N)
C5	0	0.16	300	7.6
	1	0.77	3,400 M ^a	12.9
	3	0.85	1,200 M	13.9
	7	0.86	1,600	15.5
	15	0.88	1,200	16.3
C5	0	0.14	50	6.4
	1	0.74	2,200 M	8.5
	3	0.79	1,000 M	10.4
	7	0.83	310 T ^b	11.8
	15	0.88	60 T	12.7
C3	0	0.16	300	7.6
	1	0.73	2,400 M	10.3
	3	0.82	500 T	11.3
	7	0.83	3,200	12.5
	15	0.87	250	13.0
M17	0	0.15	190	9.3
	1	0.80	2,500 M	14.4
	3	0.86	67 M	16.0
	7	0.86	200 T	17.6
	15	0.89	85 T	19.0

^a M = million

^b T = thousand

In table 1 are presented representative results showing increases in soluble nitrogen caused by *S. lactis*. In all instances the production of soluble nitrogen by the four strains of *S. lactis* tested was fairly rapid during the first day or so

when the population was at a maximum and acid production was at the most rapid rate. Later when acid production occurred very slowly or not at all and the population of viable cells was declining rapidly, the rate of production of soluble nitrogen also declined, although there was a gradual increase in the soluble nitrogen throughout the full period of experimentation. Variations in soluble nitrogen production were evident between the various strains of *S. lactis* employed and between the different trials with the same strain. The variations between different strains and variations found in three trials with one strain (C5) were of about the same magnitude.

TABLE 2

Proteolysis by Escherichia coli and Aerobacter aerogenes in heated skim milk
(av. of duplicate determinations)

Culture	Age	Titratable acidity	Plate count	Coliform count	Sol. N
	(d.)	(%)	(/ml.)	(/ml.)	(% of total N)
<i>E. coli</i>	0	0.17	180		6.7
	1	0.41		1,200 M*	5.9
	3	0.63	1,000 M	880 M	7.0
	7	0.70		400 M	7.4
	15	1.14		< 100	21.5
<i>A. aerogenes</i>	0	0.17	220		6.8
	1	0.42		2,200 M	5.8
	3	0.42		2,800 M	5.7
	7	0.46		600 M	6.4
	15	1.00	5,300	5	26.1

* M million

Representative data on *E. coli* and *A. aerogenes* (table 2) show these organisms caused a decrease in the soluble nitrogen during the first day or two when the populations were at the maxima. As the numbers of viable cells decreased these deficits gradually were overcome, and between the seventh and fifteenth days both organisms caused a marked increase in soluble nitrogen. The increase in soluble nitrogen was higher at 15 days for this strain of *A. aerogenes* than for the strain of *E. coli* used. The increase in soluble nitrogen was slightly greater during a second trial with *A. aerogenes*, while *E. coli* caused about the same increase in soluble nitrogen during each of two trials.

When *S. lactis* C6, *S. lactis* M17, *E. coli* and *A. aerogenes* were grown together in approximately equal quantities in skim milk, in no case was a rapid increase in soluble nitrogen caused by the combination of organisms (table 3). However, there was a gradual and definite rise in the soluble nitrogen in all cases, the increase being intermediate between that of the pure cultures grown singly during the early stages and more like that of *S. lactis* alone in the later stages, where the greater proteolysis typical of these gram-negative bacteria was held in check.

In one trial using the combination of organisms, extra bottles of sample were neutralized to 0.27 per cent titratable acidity with sodium hydroxide at 7 and

TABLE 3

Proteolysis by a mixture of Streptococcus lactis C5, S. lactis M17, Escherichia coli and Aerobacter aerogenes in heated skim milk (av. of duplicate determinations)

Age	Titrateable acidity	Standard plate count	Coliform count	Sol. N
(d.)	(%)	(/ml.)	(/ml.)	(% of total N)
0	0.16	160	" " " "	6.2
1	0.80	3,400 M ^a	400 M	6.7
3	0.97	600 M	20 M	8.6
7	0.85	240 M	1,300 T	11.1
15	0.94	> 300 T ^b	40	15.6

^a M = million

^b T = thousand

15 days, after which soluble nitrogen determinations were made. With the neutralized samples, the values for per cent soluble nitrogen were 11.0 and 16.6, which are not appreciably different from the values 10.7 and 16.2 which were obtained with samples which were not neutralized. Thus it seems that partial neutralization of the lactic acid formed did not influence significantly the values for protein degradation as obtained in this study.

DISCUSSION

The greatest increase in soluble nitrogen is associated with the period of rapid proliferation and acid production in the case of *S. lactis*; comparatively little increase in soluble nitrogen occurs during the period when the viable bacterial population is declining and the cells may be presumed to be undergoing some autolysis. Apparently *S. lactis* frees nitrogenous degradation products more rapidly than they can be utilized during the period of active growth. During the period of decline in population, the proteolytic enzyme system active earlier apparently becomes comparatively inactive, either because of the reduction in metabolic activity of the cells or because the reaction of the medium is not suitable for the continued action of the enzyme system concerned.

The decrease in soluble nitrogen associated with the period of maximum proliferation of *E. coli* and *A. aerogenes* indicates that utilization of the simpler nitrogenous materials is at a rate greater than that at which the cellular enzymes are acting to free such nitrogenous materials. Since the rate of increase of soluble nitrogen immediately after a maximum population has been reached continues to be slow, the early activity of the proteolytic enzymes of these organisms quite possibly is at a very low level. The considerable increases in soluble nitrogen after the cultures have been held for more than 7 days and the viable populations have declined to very low levels indicate the probability that the major proteolytic activity of these organisms is associated with cell autolysis and the consequent release of proteolytic enzymes.

The behavior of the mixed cultures during the first 7 days seems to indicate that the coliform bacteria utilize the soluble nitrogen products formed by *S. lactis* during the early stages of growth. During the period from 7 to 15 days the

presence of the *S. lactis* cells seems to retard quite markedly the proteolytic activity found to be associated with pure cultures of the coliform bacteria during this period. The lower population of coliform bacteria in the mixed culture, together with titratable acidity values slightly higher during the first 7 days of growth than would be found in a pure culture of either *E. coli* or *A. aerogenes*, seem to account for this retardation.

The increase in proteolysis which *S. lactis* alone will cause possibly is enough to demonstrate why results of some of the tests used for proteolysis in dairy products have not correlated well with other evaluations of the quality of the product. The demonstration that one organism can influence considerably the protein degradation by another organism also serves as a note of caution in the use of tests for proteolysis when mixed cultures of microorganisms are involved, as is the case in so many dairy products. These studies seem to provide additional evidence of the necessity for further studies of the influence of various microorganisms upon the enzymatic activities of other members of a mixed population.

SUMMARY

S. lactis organisms caused a rapid increase in soluble nitrogen during the first day or two, followed by a small and gradual increase which continued for at least 15 days.

When *E. coli* and *A. aerogenes* were grown alone they caused a deficit in soluble nitrogen during the first few days of growth. This deficit was overcome later, and these organisms caused marked increases in soluble nitrogen between 7 and 15 days.

Mixed cultures containing *S. lactis* and coliform organisms caused gradual increases in soluble nitrogen, increases which were somewhat between the results of the component organisms, except that the soluble nitrogen values at 15 days never were as high as those for the coliform organisms alone and were of about the same magnitude as those for *S. lactis*.

ACKNOWLEDGMENT

This work was supported in part by a grant from the Fairmont Foods Co. of Omaha, Nebr.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. *Standard Methods for the Examination of Dairy Products*. 8th ed. New York. Amer. Public Health Assoc. 1941.
- (2) BERGER, J., JOHNSON, M. T., AND PETERSON, W. H. The Proteolytic Enzymes of Bacteria. *J. Bact.*, **36**: 521-546. 1938.
- (3) BREED, E. S., MURRAY, E. G. D., AND HITCHENS, A. P. *Bergey's Manual of Determinative Bacteriology*. 6th ed. Baltimore, Md. The Williams & Wilkins Co. 1948.
- (4) HAMMER, B. W., AND PATIL, V. H. Proteolysis by *Streptococcus lactis* with Special Reference to Butter Cultures and Butter. Iowa Agr. Expt. Sta. Research Bull. 123. 1930.
- (5) KENDALL, A. I., DAY, A. A., AND WALKER, A. W. Studies in Bacterial Metabolism. *J. Am. Chem. Soc.*, **36**: 1944-1947. 1914.

- (6) LANE, C. B., AND HAMMER, B. W. Bacteriology of Cheese. I. Effect of Pasteurizing the Milk on the Nitrogenous Decomposition of Cheddar Cheese. Iowa Agr. Expt. Sta. Research Bull. 183. 1935.
- (7) SHERMAN, J. M. The Streptococci. Bact. Revs., 1: 1-87. 1937.
- (8) SPITZER, G., PARFITT, E. H., AND EPPLE, W. F. A study of the Proteolytic Action of Certain Specific Organisms on Milk Proteins in Milk and Synthetic Butter. J. Dairy Sci., 10: 15-32. 1947.
- (9) STAFFE, A. Über den Eiweissabbau in der Milch durch Bakterien der Coli-Typhus-Paratyphus-(Salomonella-)Gruppe. Milchwirtschaft. Forsch., 5: 361-373. 1928.
- (10) TAYLOR, A. E. Ueber Eiweisspaltung durch Bakterien. Z. Physiol. Chem., 36: 487-492. 1902.

THE NUTRITIVE VALUE OF TIMOTHY HAY AT DIFFERENT STAGES OF MATURITY AS COMPARED WITH SECOND CUTTING CLOVER HAY¹

N. F. COLOVOS, H. A. KEENER, J. R. PRESCOTT AND A. E. TEERI
New Hampshire Agricultural Experiment Station, Durham

It generally is recognized that certain factors such as color, stage of maturity of the plant, and texture are indicative of the feeding value of a roughage. However, little work appears to have been done to actually measure quantitatively such relationships by means of balance experiments with animals, although considerable chemical work as quoted by Huffman (6) has been carried out. Ritzman and associates, as quoted by Prince *et al.* (8), determined the digestibility of the protein and of the energy of timothy hay cut at three different stages of maturity and found that the earliest cut hay was far superior in digestibility of both protein and energy as compared to the late cut.

Newlander (7), in his review of research carried out by different agricultural experiment stations and the U. S. Department of Agriculture to determine when the hay crop should be cut, gives an excellent summary of results obtained by feeding trials. Hodgson *et al.* (5) carried out feeding trials with dairy cattle and sheep to study and compare the value of home-grown roughages, both as hays and as silages. Bohstedt (2) classified hays according to quality and determined carotene, fiber and protein content in each type of hay. Dawson *et al.* (4) determined the yield, chemical composition and feeding value for milk production of alfalfa hay cut at three stages of maturity by means of feeding trials with dairy cows.

EXPERIMENTAL

The timothy hays studied in this experiment were cut in the same field at three different stages of maturity and were cured in the field. They were compared to a second cutting mow-cured hay containing approximately 90 per cent red and ladino clovers and 10 per cent grasses. All were harvested during the summer of 1947. The timothy cut on June 21 is referred to as early timothy. It was about 16 to 18 inches in height when cut and about 10 per cent of the heads were showing. The timothy cut on July 5 is referred to as medium timothy. It was in the early bloom stage. The timothy cut on July 25 is referred to as late timothy. It was in the seed stage and many leaves were dead and brown in color. The clover hay was cut on September 9 and was approximately one-half in bloom. The hays were grown on fertile soil and the growth was moderately heavy.

The animals used to measure the nutritive value of these hays were purebred

Received for publication March 28, 1949.

¹ Contribution no. 126 of the New Hampshire Agricultural Experiment Station. The authors gratefully acknowledge the assistance of Mr. A. D. Littlehale, of the staff of this Station, in caring for the experimental animals.

dairy heifers weighing between 725 and 750 lb. at the beginning of the experiment. There were two Guernseys, one Jersey and one Ayrshire. Since 6 kg. of hay per head per day were found to be consumed completely, this amount was fed each animal in two feeds as the sole ration.

The nitrogen and energy balances were carried out between April 1 and June 12, 1948, according to the schedule given in table 1. It was planned so that each kind of hay would be fed to three different animals but to only one at a time. However, due to the refusal of the other animals to eat adequate amounts of late timothy after having been fed the other hays, it was necessary to use heifer 4 for a second balance with this hay.

TABLE 1
Schedule of balance experiments

Heifer no.	First balance	Second balance	Third balance
1	Mow-cured clover	Early timothy	Medium timothy
2	Early timothy	Mow-cured clover	(Refused late timothy)
3	Medium timothy	Mow-cured clover	Early timothy
4	Late timothy	Medium timothy	Late timothy

Although the procedure and methods do not differ materially from those used in previous research reported from this laboratory (1), a few modifications in the procedure necessitate a brief description.

Feed. The hays were chopped, mixed, sampled for analysis and weighed into separate single-feed portions before the start of the experimental period. A plain salt block was kept before each animal at all times. A preliminary feeding period of about 10 days was allowed before each of the first series of digestion balances. The length of this period later was reduced to 5 days, since all animals were on all-roughage rations.

The collection periods were from 14 to 16 days in duration. That portion of any feed refused by an animal was removed from the feed box, dried in an oven at about 55° C. and compounded. At the end of the period the total refuse was weighed at room temperature and sampled for analysis. Gross energy determinations were made on both feed and refuse by means of the bomb calorimeter. Nitrogen determinations also were made on both feed and refuse. The nitrogen and the energy in the refuse were subtracted from the amount fed in order to determine the amount consumed.

Excreta. The feces and urine were collected separately by means of the automatic collection devices developed in this laboratory. These collection devices are essentially the same as those used in this laboratory for many years (9) with the exception of minor changes in the chute. This was shortened materially in order to reduce the error due to evaporation and contamination. Both feces and urine were weighed and sampled daily. Composite samples of feces were kept at a temperature of approximately -16° C., while the composite samples of urine were stored at a temperature slightly above freezing. At the end of the collection period the feces samples were thawed and mixed

thoroughly and an aliquot was taken in triplicate for both moisture and nitrogen determinations. The remainder of the sample was dried at a temperature of about 55 to 60° C., ground in a Wiley mill and analyzed for moisture and for gross energy. The urine samples were allowed to come to a temperature of about 20° C., and their specific gravity determined; they were analyzed in triplicate for both nitrogen and gross energy. The urine samples used for the gross energy determinations were dried in the capsules under vacuum.

Metabolism measurements. At the end of each collection period, at least two 12-hour metabolism measurements were made by means of the open circuit respiration chamber which has been used in this laboratory for the past quarter century. Benedict *et al.* (1) have described this apparatus. Periodic gas recovery checks were made with the apparatus to test its accuracy. The apparatus for the analysis of the chamber air for carbon dioxide, oxygen and for the direct determination of the methane was the most recent type of the Carpenter modification (3) of the Haldane apparatus.

RESULTS AND DISCUSSION

The protein and energy contents of the hays are shown in table 2, while the nitrogen and energy balances are shown in table 3 and 4 and the metabolizable energy values in table 5.

TABLE 2
Composition of hays as fed

Hay	Moisture	Protein	Gross energy
	(%)	(%)	(Cal./g. D. M.)
Mow-dried clover	11.06	13.837	4.3895
Early timothy	10.15	7.900	4.4368
Medium timothy	9.39	6.106	4.3647
Late timothy	9.89	4.500	4.4194

The timothy hays cut at three stages of maturity show considerable variation in protein content. The late timothy contains less than one-third the protein content of the legume hay. The digestible protein picture is even more striking. The clover hay had twice as much digestible protein as the early timothy, three times more than the medium timothy and seven times more than the late timothy. The early timothy, however, gave almost as high a positive balance of protein as the clover hay because half of the gross intake of the protein in the clover hay experiments was lost in the urine. Even the late timothy supplied enough protein for maintenance. The digestibility of the protein in the timothy hay diminished appreciably as the plant advanced in maturity. The percentage digestibility decreased from 55.4 in the early timothy to 30.2 in the late timothy.

The gross energy content of all four hays studied was essentially the same. While the differences in percentage digestibility

$$\left(\frac{\text{gross energy of feed} - \text{energy in feces}}{\text{gross energy of feed}} \times 100 \right)$$

TABLE 3
Nitrogen balances

Roughage	Clover hay			Early timothy			Medium timothy			Late timothy		
	1	2	3	2	1	3	3	4	1	4	4	
Heifer no.												
Weight (lb.)	756	756	708	726	776	734	743	738	811	751	751	
Intake (g.)	131,857	132,190	132,382	74,155	75,168	75,543	54,431	57,937	57,841	40,938	42,095	
Output (g.)												
Feces	47,280	47,912	48,673	33,003	33,043	34,295	29,894	33,042	29,771	28,705	29,244	
Urine	71,302	65,760	66,273	31,500	19,327	32,532	50,823	21,839	26,057	13,870	10,722	
Total	118,582	113,672	114,946	64,503	52,370	66,827	50,717	54,881	55,828	42,575	39,966	
Balance (g.)	+13,275	+18,518	+17,436	+9,652	+22,798	+8,716	+3,714	+3,056	+2,013	-1,637	+2,129	
Digestibility (%)	64.1	63.8	63.2	55.5	56.0	54.6	45.1	43.0	48.5	29.9	30.5	
Av. digestibility ^a		63.7			55.4		45.5				30.2	

^a All differences between roughages are significant at the 1% level.TABLE 4
Energy balances

Roughage	Clover hay			Early timothy			Medium timothy			Late timothy		
	1	2	3	2	1	3	3	4	1	4	4	
Heifer no.												
Weight (lb.)	756	756	708	726	776	734	743	738	811	751	751	
Intake (Cal.)	23,289	23,302	23,304	23,421	23,768	23,810	21,661	23,473	23,650	22,800	23,483	
Output (Cal.)												
Feces	9,643	8,522	9,402	8,357	8,524	8,338	9,726	10,496	10,418	11,705	11,638	
Urine	1,454	1,164	1,091	1,473	1,045	1,003	692	696	651	526	567	
Methane	1,995	1,715	1,559	1,706	2,034	1,813	1,355	1,709	1,823	1,328	1,437	
Heat production	10,276	11,890	11,186	10,342	11,672	11,463	10,828	10,708	10,386	10,235	9,997	
Total	23,368	23,291	23,479	21,878	23,445	22,617	22,601	23,609	23,543	23,794	23,639	
Balance	-79	+11	-175	+1,543	+323	-1,193	-940	-136	+107	-994	-156	
Digestibility (%)	59.2	63.4	58.6	64.3	64.1	65.0	55.1	55.3	55.9	48.8	50.4	
Av. digestibility ^a		60.4			64.5		55.4			49.6		

^a Differences between the clover and the early timothy is not significant. The difference between the clover and the medium timothy is significant at the 5% level, while all other differences are significant at the 1% level.

TABLE 5
Metabolizable energy

Roughage	Heifer no.	Dry matter consumed	Gross energy	Metabolizable energy	Metabolizability	Metabolizable energy per g. dry matter ^a
		(g.)	(Cal.)	(Cal.)	(%)	(Cal.)
Clover hay	1	5,306	23,289	10,197	43.8	1.922
Clover hay	2	5,308	23,302	11,901	51.1	2.242
Clover hay	3	5,308	23,304	11,011	47.2	2.074
Early timothy	2	5,267	23,421	11,885	50.7	2.257
Early timothy	1	5,351	23,768	11,995	50.5	2.242
Early timothy	3	5,366	23,810	12,656	53.1	2.359
Medium timothy	3	4,967	21,661	9,888	45.6	1.991
Medium timothy	4	5,377	23,473	10,572	45.0	1.966
Medium timothy	1	5,418	23,650	10,943	46.3	2.020
Late timothy	4	5,158	22,800	9,241	40.5	1.792
Late timothy	4	5,313	23,483	9,841	41.9	1.852

^a Differences between clover and early timothy and between clover and medium timothy are not significant. Differences between early and medium timothy are significant at the 1% level and between medium timothy and late timothy at the 5% level.

were not quite as pronounced as in the case of the protein, the energy in the late timothy was 15 per cent less digestible than that in the early timothy. The early timothy hay was superior to the clover hay when the metabolizable energy values were compared. The medium timothy had much less metabolizable energy per gram of dry matter than the early timothy or the clover, while the late timothy ranked a poor fourth.

The palatability of the hays differed quite markedly. The animals cleaned up the clover and early timothy hays quite readily, but refused some of the medium timothy. In the case of the late timothy, the animals that had received the clover or the early timothy, or even the medium timothy, refused to eat very much of this poor hay. Only one animal, number 4, could be induced to eat it, probably because she had not been fed anything better than medium timothy previously.

SUMMARY AND CONCLUSIONS

The relative nutritive value of timothy hay cut at three different stages of maturity and a second-cutting mow-cured clover hay was determined by means of eleven protein and energy digestion balance experiments with dairy heifers. The early-, medium- and late-cut timothy hays contained 57.1, 44.1 and 32.5 per cent, respectively, as much protein as the second cutting clover. However, the gross energy values for all of the hays were essentially the same.

The digestibility of the protein decreased markedly from the clover hay through the different timothy hays. The values for the early, medium and late timothy hays were 87.0, 71.4 and 47.4 per cent, respectively that of the clover hay. These same hays furnished only 49.7, 31.5 and 15.4 per cent, respectively, as much digestible protein as was furnished by the clover hay.

The early-cut timothy hay was superior to the other hays with respect to

metabolizable energy. When compared to the early-cut timothy, the clover, medium timothy and late timothy contained 90.9, 87.1 and 79.7 per cent, respectively, as much metabolizable energy.

These results show that early-cut timothy may be a better source of energy than good legume hay for dairy cattle, but not of digestible protein. However, practically the same amount of nitrogen was stored from the early-cut timothy as from the clover under the conditions of this experiment. Early-cut timothy hay may furnish up to 3.2 times as much digestible protein and 1.25 times as much metabolizable energy as late-cut timothy hay.

REFERENCES

- (1) BENEDICT, F. G., COBOPACHINSKY, V., AND RITZMAN, E. G. Technik der Messung des Gesamtstoffwechsels und des Energiebedarfes von Haustieren. Abderhalden's Hand d. biolog. Arbeitmethoden, Abt. IV, Teil 13: 619-687. 1934.
- (2) BOHSTEDT, G. Hay Quality and What It Means to You. Successful Farming, June, 1946.
- (3) CARPENTER, T. M., LEE, R. C., AND FINNERTY, A. E. Ein Apparat für die exakte und schnelle Analyse der Gase aus einer Respirations Kammer. Wiss. Arch. Landw., 4, 1: 1-26. 1930.
- (4) DAWSON, J. R., KOPLAND, D. V., AND GRAVES, R. R. Yield, Chemical Composition, and Feeding Value for Milk Production of Alfalfa Hay Cut at Three Stages of Maturity. U. S. Dept. Agr. Tech. Bull. 739. 1940.
- (5) HODGSON, R. E., KNOTT, J. C., MILLER, V. L., AND MURER, H. K. The Nutritive Value of Home-grown Roughage Rations for Dairy Cattle. Wash. Agr. Expt. Sta. Bull. 366. 1938.
- (6) HUFFMAN, C. F. Roughage Quality and Quantity in the Dairy Ration: A Review. J. Dairy Sci., 22: 889-980. 1939.
- (7) NEWLANDER, J. A. When Should the Hay Crop Be Cut? Vermont Agr. Expt. Sta. Pamphlet 7. 1943.
- (8) PRINCE, F. S., BLOOD, P. T., AND PERCIVAL, G. P. Studies of Feed Value of Early Hay. N. H. Agr. Expt. Sta. Circ. 41. 1933.
- (9) RITZMAN, E. G., AND COLOVOS, N. F. An Automatic Method for Collecting Solid and Liquid Excreta from Cows in Digestion Experiments. N. H. Agr. Expt. Sta. Tech. Bull. 52. 1932.

COTTONSEED OILS IN PURIFIED AND SKIM MILK DIETS FOR CALVES¹

R. N. JARVIS AND R. K. WAUGH

Department of Animal Industry

North Carolina Agricultural Experiment Station, Raleigh

Early attempts to utilize fats other than that of milk in the diets of young calves have been reviewed by Gullickson *et al.* (5) and by Savage and McCay (7). The results have been variable and in many cases unsuccessful. Though work in recent years has shown certain fats to be more suitable than others, the reasons for the undesirability of some fats are not understood.

Gullickson *et al.* (5) observed better growth when calves received butterfat, lard or tallow than when they received liquid vegetable fats. These results suggest that a high degree of unsaturation might decrease utilization of fat and possibly other nutrients by calves. More recent studies by Jacobson and Cannon (6) and Wiese *et al.* (9) also permit this interpretation. Jacobson and Cannon (6) found that calves grew well with 3 per cent hydrogenated soybean oil in the diet. These authors also observed that calves grew fairly satisfactorily with 2 per cent crude soybean oil but not with 3 per cent. Thus it appears that there is a level above which certain unsaturated fats disrupt metabolism. Several workers have reported that calves fed liquid vegetable fats develop scours (2, 5, 6, 9). Apparently there is appreciable absorption of corn oil and cottonseed oil because calves fed these fats had higher levels of blood fat than calves receiving milk fat (5).

Observations made in the studies reported here include growth rates of calves fed purified diets as compared to whole milk, the occurrence of fatty livers in calves fed purified diets, some comparisons of cottonseed oils and butterfat for growth, and comparison of blood plasma fat levels of calves when their respective diets contain butterfat and hydrogenated cottonseed oil.

EXPERIMENTAL PROCEDURE

Animals used in these experiments were grade, purebred or crossbred dairy calves. Breed was disregarded for assignment to rations. Calves were started on the experimental rations at 2 to 4 days of age, after they had received colostrum. They were isolated in individual pens bedded with wood shavings or dehydrated sugar cane pulp. The calves were kept muzzled, but this was not wholly effective in preventing consumption of bedding. The diets were fed from nipple pails at a rate of 10 lb. daily per 100 lb. of body weight. The calves were weighed and feed allowances were adjusted weekly.

The studies reported herein were made in two trials. In trial I the diets used were (a) whole Ayrshire milk averaging 4 per cent butterfat, (b) a purified

Received for publication March 28, 1949.

¹ Approved for publication as paper no. 315 in the Journal series of the North Carolina Agricultural Experiment Station.

diet similar to that described by Wiese *et al.* (9) with 3.5 per cent refined cottonseed oil and (c) the same as diet *b* except that the oil was hydrogenated.

The purified diets differed from those used by Wiese *et al.* (9) in the following ways: (a) commercial casein² replaced vitamin-free casein; (b) calves received 0.5 minim wheat germ oil every other day in the vitamin A and D capsules but in addition each pound of milk contained 1 mg. of α -tocopherol and (c) no folic acid or biotin was added.

Trial II was a randomized block design with three diets and 15 calves. These diets were (a) whole Aryshire milk, (b) 10 per cent edible, spray-dried nonfat milk solids plus 3.5 per cent butter oil and (c) the same as diet *b* except that the fat was hydrogenated cottonseed oil.

The fat was emulsified by homogenization in all diets except the whole milk. All calves received capsules containing 30,000 I. U. of vitamin A and 1,000 units of vitamin D every other day. The sources of vitamins A and D were fish liver oil and irradiated ergosterol. Two batches of each type of cottonseed oil were used. The nonhydrogenated cottonseed oils had iodine values of 108.5 and 115.0. The hydrogenated fats had iodine values of 64.6 and 63.6 and Wiley melting points of 34.1 and 32.2° C.

TABLE 1
Daily gain and choline and lipide content of livers of calves in trial I

Diet	No. of calves	Av. time on diet	Av. daily gain	Av. liver lipides (dry basis)	Av. liver choline (dry basis)	Av. liver choline (dry, lipide free basis)
		(d.)	(lb.)	(%)	(mg./g.)	(mg./g.)
Whole milk	4	51.5	1.0 ^a	7.0	13.7	14.8
Purified basal + hydrogenated cottonseed oil	4	44.8	0.54	20.5	14.0	17.7
Purified basal + nonhydrogenated cottonseed oil	5	36.2	0.11	16.1	16.9	20.2
5% least significant difference				8.9	3.6	3.7

^a One calf sacrificed at 12 d. of age not included in this average.

All blood samples were drawn from the jugular vein. Allen's method (1) was used for the determination of plasma fat. Liver lipides were measured as the ether extract of the dried liver. Engel's method (3) was used for the determination of choline in the dry liver samples. Choline chloride was used as a standard in the determinations and all choline values are expressed as amounts of choline chloride.

RESULTS

Hydrogenated vs. nonhydrogenated cottonseed oil in purified diets. The average daily gain of calves in trial I (table 1) show that whole milk gave the best growth. Though whole milk contained on the average 0.5 per cent more

² "New Process" casein purchased from Sheffield Farms Co., Inc., 524 West 57th Street, New York, N. Y.

fat than the purified diets, this additional fat would not seem to account for the additional growth. Calves which received the purified diet with nonhydrogenated refined cottonseed oil made practically no growth, became emaciated and died in 5 to 9 weeks. These calves developed severe scours after receiving the nonhydrogenated oil about 1 week. The purified diet containing hydrogenated cottonseed oil allowed intermediate growth.

Incidence of fatty livers from purified diets. Observations on the amount of lipides in the livers of animals of trial I indicated that the purified diets containing cottonseed oil caused fatty livers (table 1). Analyses of the livers of

TABLE 2
Lipides in dried livers of calves in trial II

Diet	Lipide content in individual livers					Av.	Av. adjusted ^a
	(%)	(%)	(%)	(%)	(%)		
Whole milk	5.26	5.78	4.32	4.09	2.82	4.45	4.45
Nonfat milk solids + butter oil	3.54	8.86	3.51	5.46	19.12	8.10	5.19
Nonfat milk solids + hydrogenated cottonseed oil	5.82	6.60	4.09	5.65	6.13	5.66	5.66
5% least significant difference						5.73	1.87

^a A value of 4.62 substituted for 19.12 in the case of the animal on the butter oil diet.

the calves in trial II, in which some calves had received hydrogenated cottonseed oil in reconstituted nonfat milk solids, showed (table 2) that the cottonseed oil alone was not responsible for the fatty livers. The purified diets used in these trials apparently were responsible for the fatty livers.

TABLE 3
Average blood plasma fat and daily gain of calves in trial II

Diet	Av. plasma fat at:			Av. daily gain
	2 wk.	4 wk.	6 wk.	
	(mg./100 ml.)	(mg./100 ml.)	(mg./100 ml.)	(lb.)
Whole Ayrshire milk	156.1	164.0	180.2	.75
Nonfat milk solids + butter oil	125.8	148.3	145.9	.90
Nonfat milk solids + hydrogenated cottonseed oil	91.3	64.9	70.2	.70
5% least significant difference	57.6	57.6	57.6	.34

Blood plasma fat levels. Table 3 shows a summary of plasma fat of calves in trial II at 2, 4 and 6 weeks. The diet consisting of reconstituted nonfat milk solids and 3.5 per cent butter oil supported lower levels of plasma fat than did whole milk, but the difference was not significant. The additional 0.5 per cent fat in the whole milk might account for this difference. When hydrogenated cottonseed oil replaced the butter oil, significantly lower blood fat levels oc-

curred than on the whole milk diet at 2, 4 and 6 weeks or on the butter oil diet at 4 and 6 weeks.

The average daily gains of calves fed the different diets were not significantly different.

Liver choline. The differences in choline content of livers of calves receiving the different diets in trial I were not significant when compared on a dry weight basis (table 1). However, when the comparisons were made on a fat-free dry basis, the differences were accentuated to the extent that the average amount of choline in the livers of calves receiving whole milk was significantly greater than those receiving the purified diet with nonhydrogenated cottonseed oil.

The differences in choline content of livers of calves receiving the respective diets in trial II were not statistically significant (table 4).

TABLE 4
Choline in dried livers of calves in trial II

Diet	Choline in individual livers					Av.
	(mg./g.)	(mg./g.)	(mg./g.)	(mg./g.)	(mg./g.)	(mg./g.)
Whole milk	13.6	11.4	11.5	11.7	10.8	11.8
Nonfat milk solids + butter oil	13.5	14.3	11.7	11.2	12.5	12.6
Nonfat milk solids + hydrogenated cottonseed oil	14.0	12.8	12.5	11.6	11.5	12.5
5% least significant difference						1.1

DISCUSSION

The results obtained with hydrogenated and nonhydrogenated cottonseed oil agree with those of other workers (5, 6). Purified diets containing 3.5 per cent nonhydrogenated cottonseed oil were unsatisfactory. As Jacobson and Cannon (6) found to be the case with soybean oil, partial hydrogenation allowed calves to tolerate cottonseed oil in these studies.

The purified or synthetic diets used in these experiments did not produce as good growth as whole milk. The milk contained 4 per cent fat compared to only 3.5 per cent for the purified diets. It is not believed, however, that the additional fat in the whole milk played any important part in the results obtained. It is interesting that calves fed the more highly saturated fat in reconstituted nonfat milk solids had lower levels of plasma fat than calves receiving butter oil in the reconstituted nonfat milk solids. The hydrogenated cottonseed oil in skim milk produced growth comparable to butter oil in nonfat milk solids and to whole milk.

The cause of the high lipide content of livers of calves fed the purified diets is not known. However, since reconstituted nonfat milk solids containing hydrogenated cottonseed oil did not cause fatty liver, it seems that the purified diet was responsible. The question arises as to whether or not the purified diet contained adequate choline. Ten lb. of this diet contained 1 g. of choline chloride. Ten lb. of whole milk would contain somewhat less than this amount (4, 8); therefore a choline deficiency is not suspected.

The choline contents of the livers of the calves in these experiments were lower than has been reported previously (4, 8). The amounts of choline in the livers of calves receiving whole milk were lower than for calves which received the purified diets. The differences were not statistically significant on the dry basis, but a true difference between the whole milk diet and the purified diet containing nonhydrogenated cottonseed oil was indicated when the comparison was made on a fat-free dry basis. Inasmuch as greater differences in choline content of livers were indicated when the comparison was made on a fat-free dry basis than when on a dry basis, the question arises as to which is the proper comparison. However, if the choline content of liver is stated on a dry basis, the choline content could increase without becoming apparent if the fat content increased accordingly. Therefore it would seem to be desirable to make choline comparisons of livers on a fat-free basis when the fat content of the liver varied widely, unless total liver choline values were available.

SUMMARY

1. Calves did not grow so well when fed purified diets as when receiving whole milk. Although growing relatively poorly, the calves appeared normal when fed the purified diet containing 3.5 per cent hydrogenated cottonseed oil. They grew very poorly on such a diet containing 3.5 per cent nonhydrogenated refined cottonseed oil and died in a few weeks.

2. Calves which received the purified diets developed fatty livers whether the oil was hydrogenated or not. Since the calves receiving hydrogenated cottonseed oil with reconstituted nonfat milk solids had normal liver lipide values, it is apparent that the purified diet was concerned in the production of fatty livers.

3. The calves which received the hydrogenated cottonseed oil had lower blood plasma fat values than calves which received butterfat.

ACKNOWLEDGMENT

The authors wish to thank Dr. W. M. Roberts who made available facilities of the College Creamery; Dr. C. D. Grinnells and Prof. R. H. Ruffner, both of North Carolina State College; and John Rich, Wake Forest, who furnished calves. Procter and Gamble, Cincinnati, O., through the courtesy of Dr. W. E. Sewell, furnished the cottonseed oil. Gelatin Products, Detroit, Mich., furnished vitamin A and D capsules. Dr. H. L. Lucas, Institute of Statistics, North Carolina State College, made the statistical analyses and aided in evaluating the data.

REFERENCES

- (1) ALLEN, N. N. Blood Fat of Dairy Cattle. Minn. Agr. Expt. Sta. Tech. Bull. 130. 1938.
- (2) BATE, W., ESPE, D., AND CANNON, C. Y. Influence of Homogenization of Fat on Haircoat of Dairy Calves. *J. Dairy Sci.*, 29: 41-43. 1946.
- (3) ENGEL, R. W. Modified Methods for the Chemical and Biological Determination of Choline. *J. Biol. Chem.*, 144: 701-710. 1942.
- (4) ENGEL, R. W. The Choline Content of Animal and Plant Products. *J. Nutrition*, 25: 441-446. 1943.

- (5) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves. *J. Dairy Sci.*, 25: 117-128. 1942.
- (6) JACOBSON, N. L., AND CANNON, C. Y. Soybean Oil-filled Milks for Feeding Young Dairy Calves. *J. Dairy Sci.*, 30: 587-588. 1947.
- (7) SAVAGE, E. S., AND MCCAY, C. M. The Nutrition of Calves: A Review. *J. Dairy Sci.*, 25: 595-650. 1942.
- (8) WAUGH, R. K., HAUGE, S. M., AND KING, W. A. Choline Studies With Young Dairy Calves. I. Relation of Choline Content of Colostrum and Calf Blood. *J. Dairy Sci.*, 30: 457-461. 1947.
- (9) WIESE, A. C., JOHNSON, B. C., MITCHELL, H. H., AND NEVENS, W. B. Synthetic Rations for the Dairy Calf. *J. Dairy Sci.*, 30: 87-94. 1947.

THE AMINO ACID COMPOSITION OF BOVINE COLOSTRUM AND MILK^{1, 2}

B. C. RAY SARKAR³, R. W. LUECKE, C. W. DUNCAN AND R. E. ELY⁴
WITH THE TECHNICAL ASSISTANCE OF MRS. GERTRUDE I. WATSON
AND MRS. ALICE J. RYKALA

*Departments of Agricultural Chemistry and Dairy, Michigan Agricultural Experiment Station,
Michigan State College, East Lansing*

A review of the literature on the amino acid composition of various biological materials (3) reveals that comparatively few data are available for whole bovine milk and practically none for colostrum. However, a few workers (4, 6, 7, 13) have carried out microbiological determinations of amino acids in skim milk powder, processed milk, purified milk proteins and some of the colostrum proteins, whereas others (2, 4, 10, 16) have determined some amino acid values for milk proteins by chemical methods. Other papers, in which isolated data are given for one or more of the essential amino acids, have been reviewed by Snell (14) and Schweigert and Snell (12). Although the amino acid content of milk may be computed from the amino acid composition and the relative proportions of the various proteins occurring in it, direct determination of the amino acids in the hydrolyzate would be more desirable for routine work. In this connection Hodson and Krueger (7) determined microbiologically the essential amino acid content of fresh cows' milk. Results for a single sample of milk also have been given by Stokes *et al.* (15). The object of the present investigation was to study how the essential amino acids in colostrum and milk vary with the stage of lactation.

EXPERIMENTAL PROCEDURE

Samples of colostrum and milk were obtained from five Jersey cows and one Holstein cow from the College dairy herd maintained under winter feeding conditions. Two samples of colostrum were secured for analysis from each of the Jersey cows. The first sample was taken within 1 hour after calving and the second represented a 24-hour composite sample. Three-day composite samples of milk were taken for analysis at the 60th and 90th days of lactation. The colostrum and milk production records of the above cows were kept as a matter

Received for publication March 30, 1949.

¹ Published with the approval of the director of the Michigan Agricultural Experiment Station as journal article no. 923 (n. s.).

² This work was supported in part by a grant from the National Dairy Council on behalf of the American Dairy Association. Grateful acknowledgement also is made to Dr. Margaret A. Ohlson, head of the Department of Foods and Nutrition, for financial support in the conduct of this work.

³ Formerly visiting professor in the Department of Agricultural Chemistry. Present address: Indian Veterinary Research Institute, Izatnagar, U. P., India.

⁴ Present address: Bureau of Dairy Industry, Agricultural Research Administration, United States Department of Agriculture.

of routine procedure as well as the amount of colostrum and milk ingested by the calves during their colostrum and milk feeding periods. From these records and the percentage composition of some of the amino acids found in colostrum and milk, an approximation was made of the amount of the various amino acids ingested per day from colostrum and from milk.

The amino acid determinations were carried out microbiologically using *Lactobacillus arabinosis*, *Streptococcus faecalis* and *Leuconostoc mesenteroides*. The media used in the various determinations were essentially the same as those described by Sauberlich and Baumann (11) with the exception of those used for isoleucine and methionine, which were prepared according to the method of Kuiken *et al.* (8) and Lyman *et al.* (9). The hydrolyzates for the determination of leucine, isoleucine, valine, phenylalanine, arginine, histidine, threonine, methionine and lysine were prepared according to the method of Stokes *et al.* (15). Ten ml. of colostrum or 20 ml. of milk were taken for the digestion, the hydrolyzates finally were made up to 100 ml. and preserved with a few drops of toluene and stored in the refrigerator. For the tryptophan assay the enzymatic digestion procedure of Wooley and Sebrell (17) was followed. Five ml. of colostrum or 10 ml. of milk were used for the enzymatic determination. The digest was made up finally to 100 ml. and preserved as above. In all cases, the assays were run after the proper dilutions had been made.

In order to determine whether or not the direct hydrolysis of colostrum or milk would result in the destruction of any of the amino acids, parallel hydrolyzates of casein and of casein plus lactose were prepared and assayed for 9 amino acids. The amount of lactose used was twice the weight of the casein. To investigate this point further, the amino acid composition was determined on two samples of fresh colostrum and one sample of milk and on the precipitated proteins prepared from each colostrum and milk. The total proteins were prepared by the method of Block and Bolling (3).

The reliability and reproducibility of the various amino acid determinations used in this work were ascertained by making parallel acid hydrolyzates from the same sample of fresh unpasteurized market milk and from colostrum obtained from a first-calf Holstein cow immediately following parturition.

The total nitrogen in the colostrum and milk was determined according to the method recommended by the A. O. A. C. (1). The factor of 6.38 was used to convert nitrogen to protein.

RESULTS

Before adopting the procedure of direct acid hydrolysis of colostrum and milk, it was desirable to determine whether or not any destruction occurred in the amino acids of casein when casein was digested with 6*N* hydrochloric acid in the presence of lactose. The results obtained by the acid hydrolysis of casein, with and without lactose, are shown in table 1. By comparing the amounts of the various amino acids found in the casein by this procedure, it can be seen that the degree of destruction due to the formation of sugar-amino acid

complexes did not exceed the usual error encountered in microbiological assays. From these results it may be assumed that the amino acid composition of both albumin and globulin will remain unchanged under similar treatments. The destruction probably would be significant in the case of cystine. Therefore, the method of direct hydrolysis with 6N hydrochloric acid was followed in subsequent analyses.

TABLE 1

A comparative study of the amino acid composition of the acid hydrolyzates of casein, with and without added lactose, and of colostrum and milk and their corresponding proteins^a

Amino acid	Casein ^b without lactose	Casein with lactose	K9 colostrum	K9 protein	K12 colostrum	K12 protein	K15 milk	K15 protein
Leucine	8.11	7.89	7.27	7.36	7.52	7.35	8.64	8.55
Isoleucine	5.75	5.78	5.28	4.90	4.34	4.82	6.98	5.74
Valine	6.60	6.60	6.72	7.51	7.08	7.52	7.36	6.28
Phenylalanine	4.70	4.78	4.42	4.42	4.26	4.41	4.86	4.86
Arginine	3.79	3.41	4.64	4.90	4.77	4.61	3.64	3.51
Histidine	2.89	2.76	2.01	2.22	2.26	2.44	2.45	2.66
Threonine	4.21	4.12	6.38	6.92	6.97	7.25	4.11	4.73
Methionine	2.74	2.65	1.68	1.59	1.66	1.82	2.18	2.26
Lysine	7.07	7.13	6.62	6.79	6.98	7.05	7.75	7.96
Protein (%)	94.72	94.72	20.28	91.43	12.99	95.99	2.80	96.16

^a All values are expressed as percentage of total protein. The colostrum and milk samples were obtained from Jersey cows.

^b Vitamin-free Labco casein.

The next question that arose was that of the reproducibility of the method of microbiological assay with milk and colostrum hydrolyzates so that the determinations could be made on a routine basis. To verify this point, five aliquots from a sample of unpasteurized market milk and two aliquots from a sample of colostrum were subjected to acid hydrolysis. The results of the amino acid determinations on their hydrolyzates are presented in table 2. The

TABLE 2

Variations in the amino acid content of five individual hydrolyzates of one milk sample and two of colostrum^a

Amino acid	Fresh, unpasteurized milk						Colostrum		
	Hydrolyzate no.						Hydrolyzate no.		
	1	2	3	4	5	Av.	1	2	Av.
Leucine	8.31	8.71	8.97	8.36	8.14	8.50	7.28	7.27	7.28
Isoleucine	5.89	5.72	5.53	5.67	5.57	5.68	4.61	4.45	4.53
Valine	6.83	6.72	6.66	6.69	6.66	6.71	7.96	7.94	7.95
Phenylalanine	4.51	4.57	4.51	4.51	4.41	4.50	4.33	4.35	4.34
Arginine	3.28	3.05	2.96	3.05	3.13	3.09	4.28	4.24	4.26
Histidine	2.66	2.64	2.69	2.69	2.73	2.68	2.48	2.44	2.46
Threonine	4.83	4.80	4.61	4.73	4.92	4.78	1.95	2.02	1.99
Methionine	2.25	2.24	2.32	2.28	2.32	2.28	1.64	1.68	1.66
Lysine	7.45	7.57	7.33	7.17	7.26	7.36	6.81	7.00	6.91

^a The milk sample was fresh, unpasteurized market milk ($N = 0.505\%$) and the colostrum sample was obtained immediately after parturition from a first-calf Holstein cow ($N = 2.399\%$). All values are expressed as percentage of total protein.

maximum variation in any of the amino acids from aliquot to aliquot was within experimental error, both in case of the milk and colostrum. Based on these findings, the microbiological method was used for subsequent determinations.

In table 3 are presented the average and range of concentrations of ten amino acids in colostrum and milk samples obtained from five Jersey cows. The average protein content of the colostrum for the first- and 24-hr. samples and the milk samples obtained on the 60th and 90th days of lactation were 14.0, 10.3, 3.5 and 3.6 per cent, respectively. Inasmuch as the protein content of the colostrum taken within 1 hr. after parturition is higher than that of the 24-hr. sample, the amino acid content of the first colostrum also is considerably higher when expressed as per cent of the undried sample. However, when the amino acid composition is expressed as per cent of the protein, these differences become negligible and there is considerable overlapping in the range of values.

Individual variations were appreciably large but these were ascribed to the individuality of the cows rather than to partial destruction in hydrolysis or to errors in assay. The fact that some of the data in tables 1 and 2 differ from those in table 3 can be explained on the above basis, also on breed differences and on the plane of nutrition. Insofar as the colostrum data are concerned, the data in table 1 were obtained from the two cows of the Jersey breed, the data in table 2 were obtained from a first-calf Holstein cow, whereas the data in table 3 are the average and range of values obtained from 5 other Jersey cows. With few exceptions, the range of values presented in table 3 will include the individual values presented in tables 1 and 2. The milk data presented in table 1 were obtained from an individual Holstein cow, the data in table 2 were obtained from a market milk sample and the data in table 3 were obtained from Jersey cows at a definite stage of lactation. The cows producing the market milk undoubtedly were on various planes of nutrition, whereas all of the cows in the College herd were on the same plane of nutrition. All of the colostrum and milk data, however, are considered to be significant.

The data presented in table 1 for casein do not differ markedly from those obtained for milk (tables 1, 2 and 3). Some differences are evident because milk contains some albumin and globulin in addition to the casein.

On the average, the proteins of colostrum contain more valine, arginine, threonine and tryptophan than normal milk but less leucine, isoleucine, phenylalanine and methionine. The amounts of histidine and lysine appear to be similar in both milk and colostrum proteins. Inasmuch as the globulin content of colostrum is very high in comparison to that of milk, a difference in the amino acid composition of these two biological materials is to be expected. A comparison of the amino acid composition of the protein of bovine colostrum (table 3) with that of human colostrum (5) reveals that the protein in bovine colostrum is higher in threonine, leucine, isoleucine and valine. When the amino acid values are expressed as per cent of the sample, there is a general tendency for all of the amino acids to decline in concentration both in colostrum and milk with

TABLE 3
*Amino acid content of bovine colostrum and milk of five Jersey cows
(All values expressed as % of the undried sample and of the protein)*

Amino acid	Time after parturition				60 d.		90 d.	
	1 hr.		24 hr.		% of sample	% of protein	% of sample	% of protein
Leucine	1.36 ^a	9.71	0.90	8.83	0.36	10.29	0.36	10.04
Isoleucine	1.20-1.46 ^b	9.32-10.07	0.62-1.31	7.68-9.57	0.32-0.39	8.27-11.99	0.34-0.38	9.16-10.84
	0.81	5.85	0.63	6.28	0.25	6.96	0.25	7.05
Valine	0.75-0.87	5.22-6.30	0.51-0.77	5.77-7.85	0.21-0.29	6.62-7.65	0.24-0.27	6.33-7.77
	1.23	8.78	0.78	8.30	0.26	7.30	0.26	7.45
Phenylalanine	1.05-1.35	8.48-9.22	0.44-1.15	8.08-8.67	0.24-0.27	7.07-7.43	0.25-0.28	6.97-7.97
	0.64	4.63	0.47	4.49	0.19	5.30	0.16	4.50
Arginine	0.57-0.70	4.24-5.03	0.32-0.57	4.13-4.98	0.16-0.22	4.89-5.78	0.15-0.17	4.15-4.82
	0.74	5.31	0.51	5.04	0.13	3.78	0.14	3.97
Histidine	0.58-0.86	4.69-6.21	0.31-0.59	4.22-5.65	0.12-0.14	3.66-3.93	0.13-0.16	3.56-4.50
	0.38	2.76	0.29	2.87	0.12	2.81	0.11	3.17
Threonine	0.34-0.45	2.34-3.22	0.21-0.34	2.47-3.29	0.11-0.13	2.75-2.90	0.10-0.13	2.79-3.88
	0.97	6.93	0.61	5.96	0.17	4.96	0.16	4.53
Tryptophan	0.80-1.15	6.48-7.35	0.37-0.75	5.43-6.69	0.13-0.22	3.99-5.70	0.15-0.17	4.28-4.85
	0.25	1.81	0.24	2.29	0.05	1.46	0.06	1.70
Methionine	0.20-0.34	1.32-2.46	0.14-0.32	2.10-2.37	0.04-0.06	1.40-1.69	0.05-0.08	1.54-1.82
	0.24	1.70	0.17	1.70	0.08	2.35	0.07	1.80
Lysine	0.20-0.28	1.49-2.02	0.11-0.23	1.45-1.84	0.07-0.09	2.31-2.40	0.06-0.09	1.66-1.95
	1.06	7.56	0.72	7.15	0.27	7.79	0.24	6.75
	0.90-1.14	6.39-7.81	0.52-0.89	6.45-8.04	0.25-0.31	7.44-8.06	0.23-0.25	6.35-7.35

^a Average value.
^b Range of values.

the progress of lactation as the result of parallel diminution in the protein content.

A comparison of the amino acid composition of the milk on the 60th and 90th days of lactation reveals no significant difference except that histidine appears to increase and phenylalanine, methionine and lysine appear to decrease with the progress of lactation. The leucine, isoleucine, phenylalanine and arginine content of the market milk sample is significantly lower than that found for Jersey milk (table 3) on the 60th day of lactation. All of the other amino acids, however, seem to be comparable. A comparison of the values reported in this paper for Jersey milk with those published by Hodson and Krueger (7) reveals no significant difference with the exception of histidine, which the latter authors found to be lower.

The amount of the various amino acids secreted in the total volume of colostrum and milk at various stages of lactation and the amount of these constituents ingested by the calves during early life were calculated. Data on the output, as computed from a knowledge of the protein and amino acid contents of the colostrum and milk, are presented in table 4. The cow evidently secretes

TABLE 4

Average daily output of amino acids in the colostrum and milk of five Jersey cows and the average amounts supplied to their calves on the first day and the 60th day of lactation

Amino acid	Output			Intake	
	1st d.	60th d.	90th d.	1st d.	60th d.
	(g.)	(g.)	(g.)	(g.)	(g.)
Leucine	90.7	39.2	32.0	14.4	14.7
Isoleucine	62.9	26.7	22.5	10.0	12.2
Valine	78.5	27.9	23.7	12.5	10.7
Phenylalanine	47.6	20.3	14.3	7.6	7.1
Arginine	51.5	14.8	12.6	8.2	5.7
Histidine	28.9	10.9	10.1	4.6	4.4
Threonine	61.2	15.0	14.4	9.7	6.9
Tryptophan	23.7	5.5	5.4	3.8	2.3
Methionine	17.3	9.0	5.7	2.8	3.0
Lysine	72.4	29.8	21.7	11.5	10.6

large quantities of the various amino acids during the first 24 hr. postpartum. The output of amino acids by the 60th day of lactation shows a marked drop over that of the first day of lactation and a further small but consistent decline by the 90th day. The data on the average daily intake of amino acids by the Jersey calves on the first and 60th days of lactation are shown in the same table. These data have been calculated on the basis that the calves ingested 1.6 kg. of colostrum during the first day postpartum and 4.1 kg. of milk on the 60th day. During the first few days of life the calf ingests relatively large quantities of amino acids. By the 60th day, the total amount of amino acids obtained from milk alone did not differ markedly from that on the first day. It should be pointed out, however, that the calves were consuming appreciable amounts of dry feed at this age in addition to the milk, so the amino acid intake reported

in this paper only represents that obtained from the milk and ordinarily would be less than the total daily intake.

SUMMARY

The concentration of ten amino acids in bovine colostrum and milk has been determined.

The colostrum collected within 1 hr. after parturition is higher in total protein than the 24-hr. composite sample and thus contained larger amounts of the ten amino acids. The amino acid composition of the colostrum, based on total proteins, is similar.

No essential difference was obtained in the amino acid composition of the proteins of milk collected at the 60th and 90th days of lactation.

Data on the output of ten amino acids in colostrum and milk have been calculated and the approximate ingestion of these amino acids by the calf has been computed.

REFERENCES

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. *Official and Tentative Methods of Analysis*. 6th ed. Washington, D. C. 1945.
- (2) BEACH, E. F., BERNSTEIN, S. S., HOFFMAN, O. D., TEAGUE, D. M., AND MACY, I. G. Distribution of Nitrogen and Protein Amino Acids in Human and in Cow's Milk. *J. Biol. Chem.*, **139**: 57-63. 1941.
- (3) BLOCK, R. J., AND BOLLING, D. *The Amino Acid Composition of Proteins and Foods*. Charles C. Thomas. Springfield, Ill. 1945.
- (4) BLOCK, R. J., AND BOLLING, D. The Amino Acid Composition of Cow and Human Milk Proteins. *Arch. Biochem.*, **10**: 359-363. 1946.
- (5) BLOCK, R. J., AND MITCHELL, H. H. The Correlation of the Amino-acid Composition of Proteins with their Nutritive Value. *Nutrition Abstracts & Revs.*, **16**: 249-278. 1946.
- (6) BRAND, E., SAIDEL, L. J., GOLDWATER, W. H., KASSELL, B., AND RYAN, F. J. The Empirical Formula of *B*-Lactoglobulin. *J. Am. Chem. Soc.*, **67**: 1524-1532. 1945.
- (7) HODSON, A. Z., AND KRUEGER, G. M. Essential Amino Acid Content of Casein and Fresh and Processed Cow's Milk as Determined Microbiologically on Hydrolyzates. *Arch. Biochem.*, **10**: 55-64. 1946.
- (8) KUIKEN, K. A., NORMAN, W. H., LYMAN, C. M., HALE, F., AND BLOTTER, L. The Microbiological Determination of Amino Acids. I. Valine, Leucine and Isoleucine. *J. Biol. Chem.*, **151**: 615-626. 1943.
- (9) LYMAN, C. M., MOSELEY, O., BUTLER, B., WOOD, S., AND HALE, F. The Microbiological Determination of Amino Acids. III. Methionine. *J. Biol. Chem.*, **166**: 161-171. 1946.
- (10) PLIMMER, R. H. A., AND LOWNDES, J. Analysis of Proteins. IX. The Content in Amino Acids of the Caseinogen and Lactalbumin of Human Milk. *Biochem. J.*, **31**: 1751-1757. 1937.
- (11) SAUBERLICH, H. E., AND BAUMANN, C. A. The Effect of Dietary Protein upon Amino Acid Excretion by Rats and Mice. *J. Biol. Chem.*, **166**: 417-428. 1946.
- (12) SCHWEIGERT, B. S., AND SNELL, E. E. Microbiological Methods for the Estimation of Amino-acids. *Nutrition Abstracts & Revs.*, **16**: 497-510. 1947.
- (13) SMITH, E. L., GREENE, R. D., AND BARTNER, E. Amino Acid and Carbohydrate Analyses of some Immune Proteins. *J. Biol. Chem.*, **164**: 359-366. 1946.
- (14) SNELL, E. E. *Advances in Protein Chemistry*. II. The Microbiological Assay of Amino Acids. Pp. 85-116. Academic Press, Inc., New York, N. Y. 1945.

- (15) STOKES, J. L., GUNNESS, M., DWYER, I. M., AND CASWELL, M. C. Microbiological Methods for the Determination of Amino Acids. II. A Uniform Assay for the Ten Essential Amino Acids. J. Biol. Chem., 160: 35-49. 1945.
- (16) WILLIAMSON, M. B. The Amino Acid Composition of Human Milk Proteins. J. Biol. Chem., 156: 47-52. 1944.
- (17) WOOLEY, J. G., AND SEBRELL, W. H. Two Microbiological Methods for the Determination of l(-) Tryptophan in Proteins and Other Complex Substances. J. Biol. Chem., 157: 141-151. 1945.

THE EFFECTS OF NORDIHYDROGUAIARETIC ACID, SALT AND TEMPERATURE OF STORAGE ON THE STABILITY OF FAT AND FAT-SOLUBLE VITAMINS IN CREAM AND BUTTER¹

VLADIMIR N. KRUKOVSKY, DIONISIOS A. THEOKAS,
FRANK WHITING AND E. S. GUTHRIE

WITH TECHNICAL ASSISTANCE OF NAOMI YAGER AND MARY ANN MATTUS

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

In a recent paper (2) evidence was presented to show that the oxidized flavors in fresh milk are not associated with deterioration of fat but with the unstable lipids which are mostly a part of the stabilizing fat globule membrane. It has been pointed out, however, that the fat itself also may undergo deterioration in the presence of ascorbic acid, resulting in the development of metallic to fishy flavors and losses in vitamins A and E and carotenoids. The susceptibility of fat to this type of deterioration, as determined by the re-emulsification test (2), depends on the type of product, the temperature of pasteurization and the conditions of storage. Thus, from the biochemical point of view, at least two reactions which produce the oxidized flavors may be stimulated by the addition of ascorbic acid to milk products containing unstable fat.

Furthermore, storage tests on cream and butter (2) also have indicated that the activity of an unknown plasma factor is responsible for the sensitization of fat to the foregoing type of deterioration and that its activity is reduced to a safe minimum only in butter churned from cream pasteurized at 76.6° C.

In this connection it also is of interest to note that most of the samples of fat obtained from market butters were found unstable by the re-emulsification test (4). Although some of the butters contained added salt, there nevertheless was a possibility that the stability of fat was affected prematurely by the conditions of storage and to a lesser degree by the salt itself. A well-known factor, such as the exposure of butter to light, together with the transitional storage of butter at and above freezing temperatures, could have been the contributing factor.

Both frozen cream and storage butter are used in the preparation of foods containing ascorbic acid and possibly other substances capable of interaction with the unstable lipids. This may result in the development of the objectionable flavors and losses in the fat-soluble vitamins. Consequently, studies were conducted to learn whether the activity of the plasma factor could be restrained by the addition of a fat soluble anti-oxidant to cream.

Received for publication April 1, 1949.

¹ This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned no. 249 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

EXPERIMENTAL PROCEDURE

Five lots of cream and two lots of butter were prepared from mixed morning milk obtained from the Cornell University herd. Two portions of milk were depleted of their total vitamin C content by adding 0.03 ml. of 30 per cent H_2O_2 per l. of milk and then pasteurized at 82.2 and 87.8° C., for 30 and 10 minutes, respectively; two portions of milk containing 20 mg. of ascorbic acid per l. were pasteurized at the same temperatures and retained as the controls. Nordihydroguaiaretic acid (NDGA) anti-oxidant² was added as a propylene glycol solution at the rate of 0.005 per cent of the milk fat to a third portion of a control milk containing ascorbic acid, prior to its pasteurization at 82.2° C. Creams then were obtained from these samples.

The butter samples were churned from cream separated from milk pasteurized at 76.6° C. for 30 minutes. One part of butter was retained as a control, while the other had 2 per cent salt added.³

The samples of cream were held in tightly sealed glass containers protected from the light. The butter samples were shaped in cylindrical forms and wrapped either directly in two thicknesses of tin foil (unsalted butter) or in two thicknesses of parchment paper first and then in tin foil (salted butter). The cream and butter then were held at -17.7 to -16.1° C. for 15, 40, 76, 107, 137, 168, 198, 229 and 247 days. At the end of these periods of time, three samples from each lot of cream and butter were transferred to an incubator, the temperature of which was maintained at 0 to 1° C. and kept there for an additional 10, 20 and 30 days prior to the stability test. The cream and butter samples then were scored for flavors, and the stability of fat obtained from these products was determined by the re-emulsification test (2). Throughout the duration of this test, the samples were protected from light. They were scored for flavors; then the gravity cream was churned, and the butter obtained was centrifuged and the fat was analyzed for its fat-soluble vitamin content. Vitamins A and E and the carotenoid content of the fat were determined using Koehn and Sherman (1) and Quaife (8) methods, respectively.

RESULTS

Data on the effects of H_2O_2 treatment and the anti-oxidant activity of NDGA in cream pasteurized at 82.2° C. are presented in table 1 and figures 1, 2, 3, 4, 5, 6 and 7. Related data on cream pasteurized at 87.8° C. are not included because this temperature of pasteurization only slightly improved the stability of fat over that in cream pasteurized at 82.2° C.

The flavor scores on the graphs (figures 1 to 7) are shown by broken and solid lines which indicate the points of the development of strong metallic to fishy flavors in reconstituted milks (re-emulsification test) containing ascorbic acid alone (broken line) and together with copper (solid line) during storage for 48 hours at 0 to 5° C. These lines occasionally are preceded by short dotted lines indicating low intensity metallic-to-fishy flavors.

² Supplied by the Nordigard Corporation, Chicago, Illinois.

³ 2232 B & A, sodium chloride crystal.

TABLE 1

The effect of the elimination of vitamin C in cream, of the anti-oxidant properties of nordihydroguaiaretic acid in cream containing ascorbic acid and of 2% salt added to butter on the development of oxidized flavors.

Treatment and pastourization	-17.7 to -16.1° C.	Flavor scores ^a of cream ^b and its buttermilk after storage at						
		and then at 0 to 1° C. for						
		0 d.	10 d.		20 d.		30 d.	
		cream	cream	buttermilk	cream	buttermilk	cream	buttermilk
(d.)								
82.2° C. control	15	—	9	2	4	4	4	4
Ascorbic acid	40	—	1	1	2	3	4	4
	76	—	1	1	1	4	2	3
	107	—	4	4	3	4	3	4
	137	—	1	3	1	2	4	4
	168	—	3	4	1	4	3	4
	198	1	1	3	4	4	3	4
	229	2	4	4	4	4	4	4
	247	2	4	4	4	4	4	4

Samples of cream separated from NDGA- and H₂O₂-treated portions of milk, and the samples of salted and unsalted butter did not develop any objectionable flavors and retained their sweetness throughout the duration of the experiments.

^a— indicates no oxidized flavors detected; numbers 1 to 4 indicate increasing intensity of oxidized flavors detected.

^b 56 per cent fat.

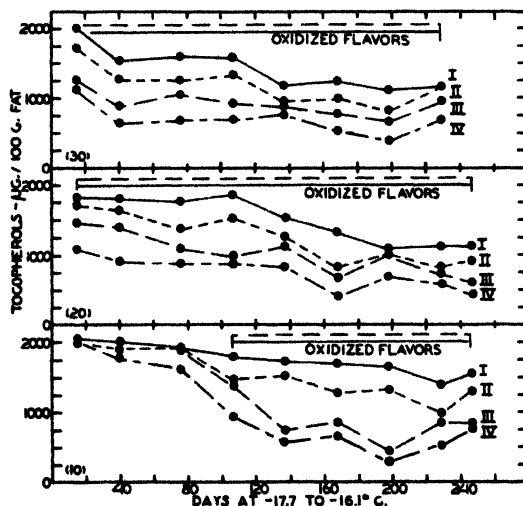


FIG. 1. The effects of holding of cream with ascorbic acid and no added anti-oxidant for 15 to 247 days at -17.7 to -16.1° C. and then for the additional 10, 20 and 30 days at 0 to 1° C. upon the development of oxidized flavors and the stability of tocopherols, as determined by the re-emulsification test. I, fat prior to re-emulsification test; II, fat from reconstituted milks containing 0.1 mg. of copper per l.; III, 20 mg. of ascorbic acid per l.; and IV, 0.1 mg. copper and 20 mg. of ascorbic acid per l.

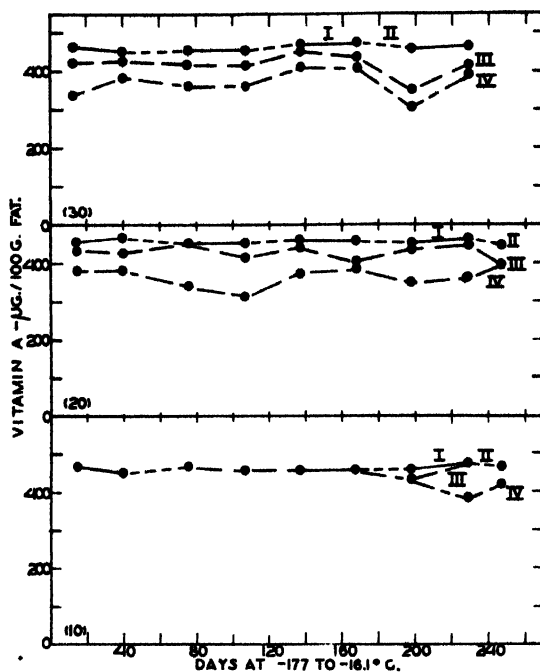


FIG. 2. The stability of vitamin A in cream with ascorbic acid and no added anti-oxidant as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

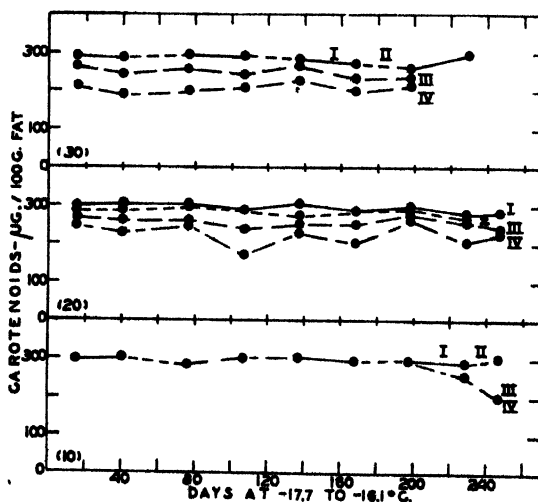


FIG. 3. The stability of carotenoids in cream with ascorbic acid and no added anti-oxidant as determined by the re-emulsification test. See figure 1 for the identification of I, II, III and IV.

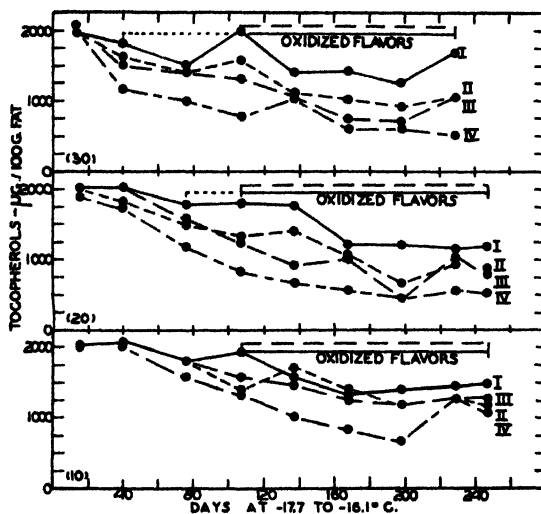


FIG. 4. The effects of holding of cream depleted of the total vitamin C content for 15 to 247 days at -17.7 to -16.1° C. and then for the additional 10, 20 and 30 days at 0 to 1° C. upon the development of oxidized flavors and the stability of tocopherols as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

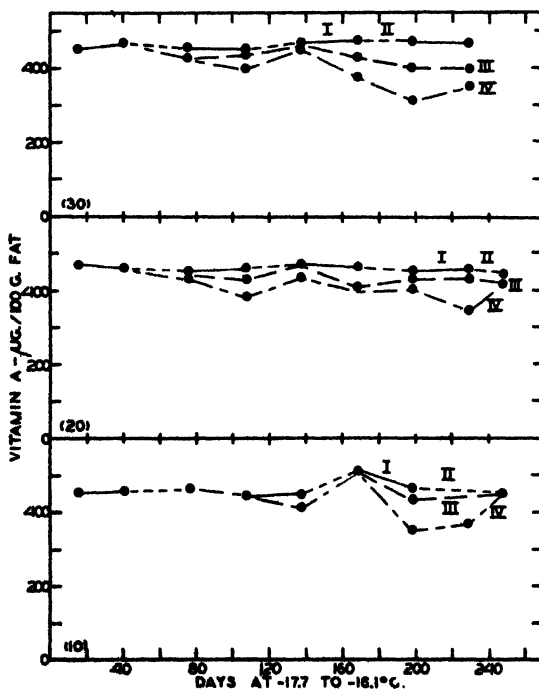


FIG. 5. The stability of vitamin A in cream depleted of the total vitamin C content as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

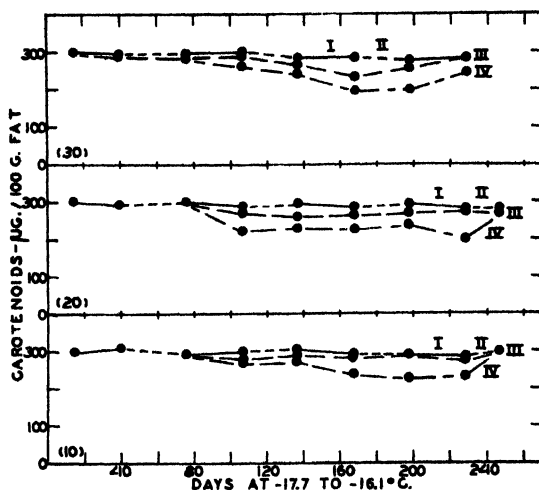


FIG. 6. The stability of carotenoids in cream depleted of the total vitamin C content as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

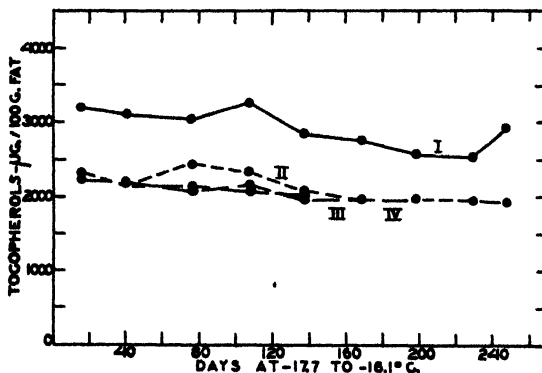


FIG. 7. The effects of holding of cream with ascorbic acid and added NDGA anti-oxidant for 15 to 247 days at -17.7 to -16.1°C . and then for the additional 10, 20 and 30 days at 0 to 1°C . upon the apparent increase of the tocopherol content of the fat, and the stability of tocopherols as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

The data of table 1 show that the depletion of cream of the total vitamin C content resulted in the prevention of oxidized flavors even when the cream was held at 0 to 1°C . after its transfer from sub-zero temperatures at the end of 15 up to 247 days of storage. The oxidized flavors were promoted in the cream separated from milk containing ascorbic acid. Their appearance and intensity varied, however, with the time of storage of cream at sub-zero, and then at 0 to 1°C . These results again show as before (2) that ascorbic acid plays an important part in the reaction which produces the oxidized flavors in cream.

The flavor scores in table 1 also revealed that NDGA anti-oxidant was as ef-

fective in the prevention of the oxidized flavors as the depletion of cream of the total vitamin C content by the rapid oxidative method, thus confirming the findings of Stull *et al.* (6). However, data in table 1 and figures 1 and 4 show that, although the elimination of vitamin C in cream by hydrogen peroxide treatment has prevented the development of oxidized flavors, the stability of fat, as determined by the re-emulsification test, was improved but slightly over that of the fat in the corresponding control cream. In this case the fat resisted deterioration for the additional 20 and 10 days only when the creams were held at 0 to 1° C. after their transfer from sub-zero temperatures at the end of storage for 15 and 40 days, respectively. As in the preceding studies (2), the susceptibility of fat to deterioration in the presence of ascorbic acid added to reconstituted milk manifested itself by the development of metallic-to-fishy flavors and losses in vitamins A and E and carotenoid content of the fat.

The data in figures 1, 2, 3, 4, 5 and 6 show that the promotion of oxidized flavors in the reconstituted milks containing ascorbic acid apparently was dependent on the stability of tocopherols and that the destruction of vitamin A and carotenoids follows that of the tocopherols. The oxidized flavors were not induced by copper alone in the reconstituted milk containing unstable fat. It caused, however, some losses in the tocopherol content of the fat. The latter was reduced appreciably when ascorbic acid acted as a catalyst alone and together with copper.

In contrast to the effects described above, the addition of NDGA to milk containing ascorbic acid resulted in the stabilization of cream against oxidized flavors and also in an apparent increase of the tocopherol content of the fat (fig. 7) and the stabilization of fat against deterioration in the presence of ascorbic acid. In this case neither the organoleptic changes nor the losses in the fat-soluble vitamins were detected, either prior to or after the re-emulsification test when applied to the fat from storage cream during the experimental trial of 277 days. Approximately 5 per cent of added NDGA was recovered in the fat churned from storage cream. This was verified by the Quaife (8) method of analysis for vitamin E, using NDGA glycol stock solution as a control. The re-emulsification test apparently removed the anti-oxidant from the fat, as evidenced by 20 to 40 per cent losses in the values for the vitamin E content of the fat.

Finally, it is of interest to point out that the addition of 2 per cent salt to butter did not affect the stability of fat stored up to 247 days at -17.7 to -16.1° C. and then for the additional 30 days at 0 to 1° C. The stability of fat held in the form of butter could be attributed to the elimination of the plasma factor described in the introductory part of this paper and to the protection of butter against the effects of the exposures to light during the storage.

DISCUSSION

The primary consideration in the selection of nordihydroguaiaretic acid (NDGA) anti-oxidant as a stabilizing agent for the fat was its solubility in fat

as described by Lundberg *et al.* (5) in their studies on the anti-oxidant properties of the compound. Although the effectiveness of this anti-oxidant in frozen cream was demonstrated by Stull *et al.* (6), it remained to be seen whether the inhibition of oxidized flavors in the cream was brought about by the stabilization of fat or plasma phases or both. There was good reason to believe (3) that the fat soluble anti-oxidant might extend a protective influence not only to the fat but also to the unstable lipids which are a part of the fat globule stabilizing membrane and that the water-soluble anti-oxidant may not stabilize the fat by virtue of the fact that it is not soluble in fat.

The data in figure 7 were conclusive in showing that nordihydroguaiaretic acid added to milk prior to pasteurization and separation of cream had penetrated the fat phase of the cream. This was evident from the apparent increase in the tocopherol content of the fat from approximately 2,000 to 3,200 γ per 100 g. of fat. Consequently, the stabilization of cream against the oxidized flavors associated with deterioration of the unstable lipids and that of the fat against the reaction responsible for the development of the metallic-to-fishy flavors and losses in the fat-soluble vitamins could be attributed to the anti-oxidant activity centered in the fat phase of the cream. The addition of NDGA to milk prevents the sensitization of the fat in cream to deterioration by a plasma factor. It is not possible to state at the present time whether the activity of the plasma factor also could be restrained by NDGA added to cream pasteurized at 61.6° C. The observations of Stull *et al.* (7) concerning the promotion of oxidized flavors in samples of cream containing both copper and NDGA, but which were pasteurized at 65° C. suggest the possibility that the anti-oxidant might not stabilize the fat in cream heated to 61.6° C. However, the addition of the anti-oxidant from glycerol solution or water suspension to cream after the pasteurization treatment (7) makes it rather doubtful whether enough time was allowed for the diffusion of NDGA into the fat phase of the cream prior to the solidification of the fat.

Since the stability of fat in butter was not as yet affected by the salt, sensitization effect of the plasma factor probably could not be attributed to the plasma salts only. Possibly substances other than salts might have been responsible for the sensitization of fat to deterioration as determined by the re-emulsification test.

It is not known whether the oxidized flavors in the reconstituted milks were associated with the formation of the new compounds resulting from the interaction of ascorbic acid and the fat-soluble vitamins, or those from the interaction of ascorbic acid and the unstable fat. The data in table 1 and figures 4, 5 and 6 (I) show, however, that in spite of the fact that the tocopherol content of the fat in cream depleted of the total vitamin C content diminished progressively with the time of storage at sub-zero and 0 to 1° C., the cream did not develop the oxidized flavors and the vitamin A and carotenoid content of the fat were not affected.

The storage tests on cream and butter, as carried on in this study, were quite

similar to the storage conditions which may be encountered in the commercial and domestic handling of these products. The data suggest that the environmental conditions adopted for this study could be applied successfully in measuring the effectiveness of various anti-oxidants.

CONCLUSIONS

Nordihydroguaiaretic acid (NDGA), added to milk at the rate of 0.005 per cent of the fat, prior to pasteurization at 82.2° C. for 30 minutes and subsequent separation, was effective in preventing oxidized flavors in cream and in stabilizing the fat and fat-soluble vitamins when the cream was held 30 days at 0 to 1° C. following storage for 15 to 247 days at -17.7 to -16.1° C.

Nordihydroguaiaretic acid caused an apparent increase in the tocopherol content of winter fat from approximately 2,000 to 3,200 γ per 100 g. of fat, indicating a possibility that the anti-oxidant activity centered in the fat phase of the cream was largely responsible for the stabilization of cream.

Depletion of the total vitamin C content of cream resulted in the prevention of the oxidized flavors for 247 plus 30 days at indicated temperatures and the fat became unstable after 30 days at 0 to 1° C. following storage for 40 days at sub-zero temperatures. In contrast to this, control cream containing ascorbic acid developed oxidized flavors during storage at both sub-zero and 0 to 1° C., and the fat became unstable after 20 days at 0 to 1° C. following storage for 15 days at sub-zero temperatures.

The fat in butter containing 2 per cent of added salt retained its stability for at least 247 days at sub-zero temperatures and then for the additional 30 days at 0 to 1° C.

REFERENCES

- (1) KOEHN, C. J., AND SHERMAN, W. C. The Determination of Vitamin A and Carotene with Photoelectric Colorimeter. *J. Biol. Chem.*, **132**: 527-538. 1940.
- (2) KRUKOVSKY, V. N., GUTHRIE, E. S., AND WHITING, F. The Stability of Milk Fat and Fat-soluble Vitamins as Determined by the Re-emulsification Test. *J. Dairy Sci.*, **31**: 961-972. 1948.
- (3) KRUKOVSKY, V. N., LOOSLI, J. K., AND WHITING, F. The Influence of Tocopherols and Cod Liver Oil on the Stability of Milk. *J. Dairy Sci.*, **32**: 196-201. 1949.
- (4) KRUKOVSKY, V. N. Unpublished data. Ithaca, N. Y. 1947.
- (5) LUNDBERG, W. O., HALVORSON, H. O., AND BURR, G. O. The Anti-oxidant Properties of Nordihydroguaiaretic Acid. *Oil & Soap*, **21**: 33-35. 1944.
- (6) STULL, J. W., HERREID, E. O., AND TRACY, P. H. A Study of the Use of Nordihydroguaiaretic Acid in the Storage of Frozen Sweet Cream. *J. Dairy Sci.*, **30**: 541. 1947.
- (7) STULL, J. W., HERREID, E. O., AND TRACY, P. H. A Study of the Use of the Anti-oxidant Nordihydroguaiaretic Acid in Dairy Products. II. Its Antioxygenic Properties in Unsweetened Frozen Cream. *J. Dairy Sci.*, **31**: 1024-1028. 1948.
- (8) QUAIFFE, M. L. Tocopherols (Vitamin E) in Milk: Their Chemical Determination and Occurrence in Human Milk. *J. Biol. Chem.*, **169**: 513-514. 1947.

OCURRENCE IN BLOOD PLASMA FROM CERTAIN DAIRY CALVES OF FACTORS THAT INTERFERE WITH THE COLOR REACTIONS OF ACTIVATED GLYCEROL DICHLOROHYDRIN WITH VITAMIN A AND CAROTENOIDS¹

R. S. ALLEN, G. H. WISE AND N. L. JACOBSON

Iowa State College, Ames

The introduction of a new chemical method of assaying blood for vitamin A presents the problem of adaptation to different species and to the same species under different dietary conditions. Activated glycerol dichlorohydrin (GDH), recently introduced by Sobel and Werbin (10, 11) as a colorimetric reagent that has certain advantages over the usual antimony trichloride reagent, has been used successfully for the measurements of vitamin A and carotene in the blood serum of human subjects (9) and of vitamin A in blood plasma of dairy cows (12).

Abnormally low vitamin A values occasionally were observed in the plasma of certain calves in the Iowa State College dairy herd when the Kimble procedure (5) was used for extracting vitamin A and carotenoids and GDH was employed as the colorimetric reagent. In view of factors affecting the vitamin A color reaction with antimony trichloride (2, 3, 6, 7, 8), it was postulated that the aforementioned low values were due to the presence of some substance or substances that interfere with the color reaction with GDH. Although this difficulty has not been reported for blood plasma, Wall and Kelley (13) found that petroleum ether extracts of fortified poultry mashes contained substances that suppress the GDH-vitamin A color development.

The primary objective of this study was to modify the recommended GDH procedure to overcome the difficulties occasionally encountered in vitamin A analysis of blood plasma from dairy calves.

EXPERIMENTAL

Analytical methods. Since saponification of blood serum has been used as a means of counteracting the effects of inhibitors of the antimony trichloride-vitamin A reaction (1, 8), the logical step was to compare saponification and non-saponification using GDH as the colorimetric reagent for vitamin A and carotene. The detailed methods, employing the Beckman spectrophotometer for colorimetric readings, were as follows:

a. Nonsaponification method. The method of extracting calf blood plasma was essentially the same as that of Kimble (5). Nine ml. of blood plasma² were pipetted into a 50-ml. glass-stoppered centrifuge tube to which were added 9 ml. of 95 per cent ethanol and 21.6 ml. of redistilled Skellysolve A. The tube was stoppered and shaken by end-over-end inversion at the rate of 100 times per

Received for publication April 2, 1949.

¹ Journal paper no. 1630 of the Iowa Agricultural Experiment Station. Project no. 814.

² Since samples of calf plasma often contain relatively small quantities of vitamin A and carotene, the quantity was increased to 9 ml. to improve the accuracy of the analyses.

minute for 10 minutes. Subsequently, the tube was centrifuged at low speed for approximately 30 seconds to obtain a clear supernatant layer of Skellysolve A extract, 18 ml. of which then were transferred to another 50-ml. centrifuge tube.

The solvent was evaporated by warming the tube in a water bath at 45 to 55° C.; when most of the solvent had been removed, a stream of nitrogen was passed over the extract until evaporation was completed. The tube was stoppered and cooled to room temperature after which 1.0 ml. of redistilled U.S.P. chloroform (dried over anhydrous Na_2SO_4) was added to dissolve the dried extract. Four ml. of GDH were added and mixed thoroughly with the chloroform solution.

Approximately 3 ml. of the resulting solution were poured into a 1-cm. Beckman Corex cell for the determination of the optical density of the colors resulting from the interactions of the vitamin A and the carotenoids with GDH. The optical density measurements were made in a Beckman model DU spectrophotometer with the slit width set at 0.065 mm. Two minutes after the addition of GDH, readings were made at a wave length of 555 $m\mu$ for vitamin A and 4 minutes after the addition, at 950 $m\mu$ for carotenoids. The instrument was set at 100 per cent transmission, using a solution containing 4 ml. of GDH and 1 ml. of chloroform.

The Beckman spectrophotometer was standardized at 555 $m\mu$ using U.S.P. Vitamin A Reference Standard (crystalline vitamin A acetate in cottonseed oil) and at 555 and 950 $m\mu$, using crystalline β -carotene^a according to the method of Sobel and Snow (9).

The carotenoid determination at 830 $m\mu$ with GDH is reported to be equally as sensitive as the usual method of determination in the Skellysolve A extract at 440 $m\mu$ (9). Preliminary observations revealed that the sensitivity of the measurement of carotene with GDH as the colorimetric reagent steadily increased between 800 and 1000 $m\mu$. In view of these findings and in order to increase the sensitivity of carotene measurements in calf blood plasma, a wave length of 950 $m\mu$ was selected. Carotenoid values in calf plasma measured at 440 $m\mu$ in Skellysolve A extract were in agreement with those measured at 950 $m\mu$ using GDH as the colorimetric reagent, but the latter procedure is more convenient when the Beckman spectrophotometer is employed.

b. Saponification method. This procedure was essentially the same as the foregoing except that 9 ml. of freshly prepared 1 N KOH in 95 per cent ethanol were substituted for 9 ml. of 95 per cent ethanol. Although the use of 1 N KOH in 90 per cent ethanol has been recommended for saponification of human serum (1, 9), preliminary observations indicated that 1 N KOH in 95 per cent ethanol was a satisfactory solution for saponification of calf plasma. After the addition of the alcoholic KOH, the tube was stoppered and the contents were mixed thoroughly. The stopper was loosened and the tube was placed into a 60° C. water bath for 20 minutes. The plasma thus saponified was cooled to room temperature and 21.6 ml. of redistilled Skellysolve A were added. These steps in the saponification were essentially the same as those described by Sobel and Snow (9). Subsequent steps were the same as described in the nonsaponification

^a Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

method. No significant differences were observed when aldehyde-free 95 per cent alcohol was substituted for U.S.P. alcohol in the saponification procedure. Blank runs were negative.

Validity of procedures. Internal standards were used as a means of estimating the accuracy of the respective analytical procedures and of ascertaining whether the difficulty was due to inefficient extraction or to the presence of a suppressing agent. These standards were prepared at frequent intervals by dissolving, respectively, known quantities of U.S.P. Vitamin A Reference Standard and crystalline β -carotene in redistilled U.S.P. chloroform. One ml. of standard solution was substituted for the chloroform in the foregoing procedures. The dried extracts of both saponified and nonsaponified plasma samples were dissolved in the internal standard solution and then analyzed for the total vitamin A and carotenoid content.

Blood samples. Venous blood samples were drawn from calves of various ages and on several different diets. The plasma was separated by centrifugation of oxalated blood, and most analyses were made within 48 hours after collection.

RESULTS AND DISCUSSION

Effect of color suppression on accuracy of the procedures. The results from the use of vitamin A internal standards in the analysis of calf plasma are summarized in table 1. The recovery of added vitamin A in the nonsaponified plasma

TABLE 1

Recovery by the GDH method of known amounts of vitamin A added to extracts of nonsaponified and of saponified blood plasma from calves

Calf no.	Nonsaponified			Saponified		
	Original	Internal standard		Original	Internal standard	
		Added	Recovered		Added	Recovered
	($\gamma/100$ ml.)	(%)		($\gamma/100$ ml.)	(%)	
3129	21.2	62.8	56.4	21.0	62.8	99.4
3140	5.8	32.4	16.7	25.1	32.4	95.4
3142	21.0	62.8	60.2	21.8	62.8	99.2
3148	20.3	30.1	94.7	21.8	30.1	100.7
3151 ^a	0.8	68.0	0.6	17.2	68.0	89.9
3151 ^b	0.1	33.9	10.6	15.7	33.9	96.2
3152	14.5	33.8	85.8	16.4	33.8	93.8
3156	1.3	32.7	15.9	8.4	32.7	87.3
3160	18.6	32.4	79.3	20.7	32.4	105.6
3167	9.7	31.4	65.6	13.0	31.4	91.4
3168	1.0	31.4	9.2	8.5	31.4	90.1
Pooled ^c	13.1	23.9	68.2	14.9	23.9	89.1

^a Sample collected 1/13/49.

^b Sample collected 1/18/49.

^c Samples from several calves.

ranged from 0.6 to 94.7 per cent while that in the saponified samples varied from 87.3 to 105.6 per cent. As a matter of convenience, the quantities of vitamin A added were not standardized; this, however, does not vitiate the comparative results from nonsaponification and saponification. In all instances, the recovery of

added internal standards was better from the latter than from the former on the same sample of plasma, thus indicating that the abnormally low values occasionally noted in calf blood plasma probably were due more to suppressing agents than to incomplete extraction of vitamin A.

Preliminary experiments indicated that the recovery of β -carotene added to the extracts of saponified blood plasma was better than from nonsaponified plasma. It is possible that the initial level of carotenoids and/or dietary factors other than the carotenoid intake may be involved.

In accord with observations from the use of antimony trichloride reagent (8), color development with GDH in certain nonsaponified samples was slow. The maximum absorptions at 555 $m\mu$ and at 950 $m\mu$ were not attained until 4 to 8 minutes and 8 to 10 minutes, respectively, after addition of the GDH. These absorption maxima after the protracted times were not so great as those obtained when the samples were saponified and measured at the prescribed periods, 2 and 4 minutes, respectively. Apparently substances having a depressing effect on the rate as well as on the extent of color development with vitamin A and with carotene were present.

Although the chemical nature of the inhibitory constituents of the blood plasma of calves has not been determined, these factors may be the same as or similar to the lipidlike substances (8) interfering with the antimony trichloride-vitamin A color reaction.

The wide range of recovery of added vitamin A indicates considerable variability in the quantity of inhibitors present. In half of the samples, recoveries of internal standards from extracts of saponified plasmas were less than 95 per cent, suggesting that this treatment did not counteract or remove completely the color inhibitors. It is possible that the time and/or temperature employed in saponification may be adjusted to overcome completely the color suppression. The consistently higher percentage recoveries affected by the present saponification procedure render it preferable to the usual nonsaponification method.

Factors possibly related to the suppression of color development. Among several factors that may be related to the presence of factors interfering with color development, diet seemed to warrant consideration. With this in view several dietary groups were studied. The analytical results presented in table 2 indicate that the greatest degree of suppression of the vitamin A color reaction was in calves of group I, which received whole milk daily and 100,000 I.U. of either carotene or vitamin A per 100 lb. body weight at bi-weekly intervals. Saponification effected an increase not only in vitamin A values but also in the carotenoid concentrations, the former being more marked than the latter. The color inhibition varied with different individuals; calf 3151 consistently had lower vitamin A levels than other calves when the plasma was not saponified. In another experiment blood samples were drawn from this subject at 3-hour intervals over a 15-hour period following the administration of 100,000 I.U. of natural ester vitamin A per 100 lb. body weight. When the nonsaponification procedure was used, no vitamin A was detected in the blood plasma, but when

saponification was employed, the vitamin A levels in the sequential collections were 17.2, 18.5, 22.7, 29.2, 23.1 and 19.3 γ per 100 ml. plasma.

The diets of the calves in group II included hay, hence the high carotenoid levels. These values were affected to a greater extent by saponification than

TABLE 2

Vitamin A and carotenoids, as determined by the GDH method, in nonsaponified and in saponified samples of blood plasma from calves receiving various diets

Dietary group	Calf no.	Vitamin A		Carotenoids	
		Nonsaponified	Saponified	Nonsaponified	Saponified
(γ/100 ml.)					
I Whole milk with occasional vitamin A or carotene supplement	3151 ^a	0.0	14.5	15.1	18.0
	3151 ^a	0.1	15.7	11.2	13.5
	3151 ^a	0.8	17.2	15.0	19.4
	3156	1.3	8.4	8.7	10.9
	3167	9.7	13.0	10.5	12.5
	3168	1.0	8.5	9.4	12.4
II Reconstituted butter-milk, hay and concentrate mixture	3129	21.2	21.0	75.3	80.1
	3140	5.8	25.1	88.9	113.2
	3142	21.0	21.8	94.0	108.8
	3148	20.3	21.8	35.4	38.6
	3152	14.5	16.4	32.3	40.5
	3160	18.6	20.7	54.7	64.8
III Concentrate mixture plus daily supplement of 5,000 I.U. carotene/100 lb. body wt.	2991	5.2	5.1	37.8	39.1
	2994	7.2	8.2	21.3	21.2
	3007 ^b	10.2	9.9	46.5	45.3
	3007 ^b	7.8	7.8	35.1	33.3
	3007 ^b	5.1	5.5	31.8	34.9
IV Concentrate mixture plus daily supplement of 10,000 I.U. vitamin A/100 lb. body wt.	2991 ^b	35.2	35.5	21.2	22.8
	2991 ^b	45.4	42.5	13.6	14.2
	2994 ^b	44.3	50.4	21.3	20.3
	2994 ^b	62.4	65.3	17.3	16.4

^a Three samples drawn over a 6-week period.

^b Samples taken at weekly intervals.

were those for vitamin A. Calf 3140, however, was a notable exception, thus further emphasizing the role of individuality.

The differences in vitamin A and in carotenoid levels found by the two chemical methods were insignificant in groups III and IV, thus indicating little or no action of inhibitors on color development. Although the experimental subjects of these two groups were older than those of the other groups, it is improbable that age *per se* was related to group differences in color suppression.

The foregoing results suggest that dietary constituents may be important factors relating to the presence of inhibitory substances in calf blood plasma. Inasmuch as milk was common to all calves in which the color suppression was noted, it seems possible that this dietary constituent may be an etiological factor. However, in view of the limited observations and the possible interrelationship of other factors, the present data can be considered merely as indicative.

The data from group I suggest the possibility that occasional administration of massive amounts of certain types of concentrates of vitamin A and/or carotene intensifies the suppression effect.

If diet affects the presence or activity of inhibitors, this may account for some of the reported irregularities of vitamin A levels in calf blood. Jacobson and Thomas (4) found a decided increase in the plasma vitamin A level when calves that had received massive amounts of vitamin A daily were placed on a deficiency diet.

It should be emphasized that individual metabolic idiosyncrasies seem to play a more pronounced role than other factors that have been considered. The results presented herein point to the need for further exploration of the factors affecting the apparent concentrations of vitamin A and carotene in the blood of calves.

SUMMARY

Samples of blood plasma from calves were analyzed for vitamin A and carotenoids by saponification and by nonsaponification methods using activated glycerol dichlorohydrin (GDH) as the colorimetric reagent.

The accuracy of analysis, as measured by the use of internal standards, was increased by saponification of plasma.

Low vitamin A values occasionally found by the nonsaponification method are attributed primarily to the presence of inhibitors of color reactions.

Color suppressing factors apparently contributed to the low carotenoid levels found in some nonsaponified samples of calf blood plasma.

Although a marked variability among individual calves was noted, there appears to be a possible relationship between diet and the presence of color inhibitors in blood plasma.

Since there is no known method of predicting the presence of substances that suppress the color reactions, it is recommended that all calf blood plasma samples be analyzed by the saponification method when GDH is used as the colorimetric reagent.

ACKNOWLEDGMENT

The authors wish to express their appreciation for the helpful suggestions given by Dr. S. W. Fox, Iowa State College, in the investigation, and Dr. D. B. Parrish, Kansas State College, in the preparation of the manuscript.

REFERENCES

- (1) BESSEY, O. A., LOWBY, O. H., BROCK, M. J., AND LOPEZ, J. A. The Determination of Vitamin A and Carotene in Small Quantities of Blood Serum. *J. Biol. Chem.*, **166**: 177-188. 1946.
- (2) CORBET, R. E., GEISINGER, H. H., AND HOLMES, H. N. Substances which Interfere with the Antimony Trichloride Test for Vitamin A. *J. Biol. Chem.*, **100**: 657-666. 1933.
- (3) HEILBRON, I. M., GILLAM, A. E., AND MORTON, R. A. Specificity in Tests for Vitamin A. A New Conception of the Chromogenic Constituents of Fresh and Aged Liver Oils. *Biochem. J.*, **25**: 1352-1366. 1931.

- (4) JACOBSON, W. C., AND THOMAS, J. W. Some Irregular Fluctuations in the Vitamin A Level of Blood Plasma Produced by Ration Changes in Calves. *J. Dairy Sci.*, **31**: 671-672. 1948.
- (5) KIMBLE, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, **24**: 1055-1065. 1939.
- (6) NORRIS, E. R., AND CHURCH, A. E. A Study of the Antimony Trichloride Color Reaction for Vitamin A. *J. Biol. Chem.*, **85**: 477-489. 1929-30.
- (7) NOTEVAEP, O., AND WEEDON, H. W. Spectrographic Studies on the Antimony Trichloride Reaction for Vitamin A. III. The Relation of the Spectral Absorption of the Blue Solutions of Oils to that of their Concentrates. *Biochem. J.*, **32**: 1668-1674. 1938.
- (8) PARRISH, D. B., WISE, G. H., AND HUGHES, J. S. Vitamin A and Carotenoids in the Blood Serum of Dairy Cattle. *Anal. Chem.*, **20**: 230-233. 1948.
- (9) SOBEL, A. E., AND SNOW, S. D. The Estimation of Serum Vitamin A with Activated Glycerol Dichlorohydrin. *J. Biol. Chem.*, **171**: 617-632. 1947.
- (10) SOBEL, A. E., AND WERBIN, H. Activated Glycerol Dichlorohydrin. *Ind. Eng. Chem., Anal. Ed.*, **18**: 570-573. 1946.
- (11) SOBEL, A. E., AND WERBIN, H. Determination of Vitamin A in Fish Liver Oils with Activated Glycerol Dichlorohydrin. *Ind. Eng. Chem., Anal. Ed.*, **19**: 107-112. 1947.
- (12) SQUIBB, R. L., CANNON, C. Y., AND ALLEN, R. S. Effect of Raw Soybeans and of Soybean Oil on Plasma Carotene and on Vitamin A as Measured by Activated Glycerol Dichlorohydrin. *J. Dairy Sci.*, **31**: 421-427. 1948.
- (13) WALL, M. E., AND KELLEY, E. G. Determination of Vitamin A Ester in Fortified Poultry Mash with Activated Glycerol Dichlorohydrin. *Anal. Chem.*, **20**: 757-759. 1948.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

AUGUST, 1949

NUMBER 8

ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-FOURTH ANNUAL MEETING

MANUFACTURING SECTION

M1 Body of Cultured Cream. E. S. GUTHRIE, Cornell University.

Pasteurization temperatures near 165° F., with a holding period of 30 min., gave the smoothest, driest, and most viscous body in the final product. Homogenization pressures of approximately 3,000 lb. to the in.² resulted in the smoothest, driest and most viscous body when homogenized in a single stage. Rehomogenizing at pasteurization temperatures, or a few degrees below, increased the firmness, dryness and viscosity of the body. A total of 5,000 or 6,000 lb. pressure to the in.² in both stages apparently was the limit. Above those pressures the cultured cream was grainy and showed some whey.

The use of rennet made a firmer and more viscous body than when rennet was not employed. Its adaptability is limited by regulations of boards of health.

M2 The Anti-oxidant Properties of Nordihydroguaiaretic Acid in Cream. V. N. KRUKOVSKY, D. A. THEOKAS, AND F. A. WHITING, Cornell University.

Storage tests on frozen cream and butter indicated that apparently the activity of an as yet unknown plasma factor was responsible for the sensitization of fat to deterioration which, in the presence of ascorbic acid, manifests itself by the development of metallic-to-fishy flavors and losses in content of vitamins A and E in the fat. Experiments were performed, therefore, to learn whether the addition of the fat-soluble anti-oxidant nordihydroguaiaretic acid (NDGA), to cream would result in the stabilization of fat against the foregoing type of deterioration, under the environmental conditions which render fat unstable in a comparatively short time. NDGA was added at the rate of 0.005% of the bulk fat to milk prior to pasteurization at 82.2° C. for 30 min. and separation. The stability of fat was determined in cream held up to 30 d. at 0 to 1° C. following its storage for 15 to 247 d. at sub-zero temperatures, using the re-emulsification test.

NDGA anti-oxidant was effective both in preventing the oxidized flavors in cream, and in the stabilization of fat and the fat-soluble vitamins during storage for 247 + 30 d. NDGA also caused an apparent increase in the tocopherol content from approximately 2,000 γ (winter fat) to 3,200 γ per 100 g. of fat, suggesting a possibility that the anti-oxidant activity centered in the fat phase of the cream was responsible largely for its stabilization. In contrast to this, the oxidized flavors were promoted in the control cream after 10 d., and the fat became unstable after 20 d. at 0 to 1° C., following storage for 15 d. at sub-zero temperatures.

M3 The Relation Between The Degree of Solidification of Fat in Cream and Its Churning Time. J. R. BRUNNER, Michigan State College, AND E. L. JACK, University of California.

The degree of solidification of the fat in cream at the time of churning is measured by a thermal method (J.D.S., 26: 169. 1943). Experimental results seem to indicate that the portion of fat solidified in creams that churn in 40 to 45 min. range from 20 to 35% in creams cooled from the temperature of pasteurization to the churning temperature and from 55 to 65% for creams held cold and then warmed to the churning temperature. In general the fat losses in the buttermilk were greater when the first cooling procedure was used, whereas the employment of the second cooling procedure resulted in the production of butter with a tendency toward crumbly body defect.

M4 The Stability to Drying of Added Vitamin A to Spray Dried Milk. F. C. OLSON, G. W. GRUBER, R. KOZLIK, AND K. BROWN, Maple Island Farm, Inc. Stillwater, Minn.

Vitamin A ester concentrate was added to milk before drying in concentrations of 750 to 20,000 U.S.P. units/g. of dry powder. Vitamin A was determined by the glyceryl dichlorhydrin method on the condensed milk and dry powder. When

an emulsifier is added to the vitamin A oil so as to mix the vitamin A thoroughly, there is no loss of Vitamin A in drying.

M5 The Effect of Variations in Acidity on the Keeping Quality of Dried Milk. G. R. GREENBANK AND P. A. WRIGHT, Bureau of Dairy Industry, USDA.

Theoretically the natural antioxidants in milk may be regenerated by the addition of a proton after they have become inactive or reduced in activity. The protons may be supplied by acids. Lactic acid was used in these experiments and was added to the raw milk before processing. The control sample was processed in the same manner, but the milk was not acidified. The maximum pH that will increase the keeping quality of the dried milk is being studied. The decrease in pH necessary to promote better keeping quality is not detectable by the average person. The acidified samples had better keeping quality.

Evacuation of container and release of vacuum with nitrogen, evacuation and release with CO₂, canned samples held under high pressure of CO₂, pressure being released before closing the cans and cans held under pressure of CO₂ and then evacuated and the vacuum released with nitrogen have been compared. Those samples packed by the third method had much better keeping quality than the others. This may be the result of the action of CO₂ as an acid or the decrease of partial pressure of oxygen in the fat. The exact cause is being studied.

M6 A Method of Measuring Ice Crystal and Air Cell Size of Ice Cream by Microscopical Examination. L. F. BLANTON AND W. S. ARBUCKLE, North Carolina State College.

A rapid, accurate method for measuring ice crystal and air cell size of ice cream has been developed. The method involves the embedding of thin sections of ice cream in an oil with a refractive index of 1.420 and examining microscopically at -15° F. at a magnification of 100 times. The microscopical field is projected on a ground glass screen and measurements of the length and width of air cells and ice crystals are taken with a millimeter scale and converted to microns. Statistical analysis has been made on the data taken from a number of similar mixes frozen on three different freezers and an experimental design for measurement studies is proposed.

M7 The Use of Whey in Sherbets.¹ F. E. POTTER AND D. H. WILLIAMS, Bureau of Dairy Industry, USDA.

Swiss, Cheddar and cottage cheese whey, as well as sweetened and plain condensed Swiss or

Cheddar whey, and dehydrated whey were used in sherbets. Sherbets from concentrated wheys were made to contain 5 per cent whey solids, and the addition of sugar, stabilizer and flavor produced a sherbet containing 4.25 per cent whey solids.

The sherbets could be frozen to any desired overrun on the continuous freezer, but with the batch freezer overruns greater than 70 per cent were obtained before the sherbet was frozen properly. Removal of a major fraction of the protein through heat coagulation did not materially alter whipping properties. Addition of more than 0.6% fat to the whey sherbet formula reduced the overrun to less than 50% when frozen on the batch freezer. The homogenization of whey sherbets containing fat increased the overrun.

Sherbets from cottage cheese whey had an acceptable titratable acidity of 0.35% but sherbets from low acid whey had approximately the same titratable acidity as milk sherbets and therefore required the addition of citric acid.

Sherbets of 1 or 2 per cent butterfat and 5 per cent whey solids could not be distinguished consistently from ice cream mix sherbets when whey of a good quality was used. The whey sherbets were more refreshing than the sherbets from ice cream mix.

M8 The Effect of Some Emulsifying Agents on the Physical-Chemical Properties of Ice Cream. J. J. SHEURING, University of Georgia, Athens, H. PYENSON AND P. H. TRACY, University of Illinois.

A standard mix was prepared, frozen in a 40-quart batch freezer, packaged in pint paper cartons and stored at -15 and 5° F. One set of samples was heat-shocked by placing them at 40° F. for 0.5-hr. periods weekly for 4 weeks and then returning to the storage cabinet each time. Sorbitan monostearate, glycerol monostearate and a mannitol ester of stearin were studied with and without the use of gelatin as a stabilizer.

In the amounts used in ice cream, the emulsifying agents were not detected by modified Babcock procedures but were detected in most cases by ether extraction methods. They have no preservative action, produce no marked differences in pH, acidity and viscosity in ice cream mixes when used alone or in combination with gelatin. Heat-shocking of samples containing emulsifying agents and storing at elevated temperatures (5° F.) reduced body and texture scores to about the same degree. The emulsifying agents did not offer any protection against heat-shocking.

¹This work was done with funds from the Agricultural Research and Marketing Act of 1946.

M9 Some Factors Influencing Shrinkage in Ice Cream. J. J. SHEURING, University of Georgia.

This study was undertaken to investigate the effect of air cell size, ice crystal structure, protein destabilization, external and internal pressures, temperatures of storage and ice cream porosity upon shrinkage. Although the study is not complete, the following information has been obtained: (a) ice cream shrinkage is not due to decrease in air cell size due to lowering temperatures, (b) porous ice cream does not shrink as much as a non-porous product, (c) shrinkage probably is caused by collapse of air cells due to changes in external pressure and (d) the internal pressure of ice cream does not change noticeably during the hardening process.

M10 The Manufacture of "Cultured" Ice Cream. W. H. E. REID, J. H. GHOLSON, C. B. AGEE, AND R. M. HANCKEL, Missouri Agricultural Experiment Station, Columbia.

Variable amounts of dehydrated culture do not present special problems in calculating, processing, freezing of the mix or hardening of the ice cream. The distinctive cultured flavor becomes more pronounced with increased amounts of the dehydrated culture. With increased increments of the dehydrated culture, the body becomes more smooth and mellow, the texture becomes proportionally closer and the resistance to melting greater. Three per cent of culture seems to be most desirable to the majority of the consumers, 5% resulting in a slight powdery feel in the mouth and a cultured flavor which may be too pronounced for some consumers. The resultant ice cream shows slight excessive stability and has a tendency to be brittle. As the percentage of butterfat is increased, the cultured flavor tends to be submerged.

The general trend, when increasing the amount of dehydrated culture in vanilla ice cream, was an increase in the stability of the ice cream with increased increments of dehydrated culture. By replacing one-third of the sucrose with dextrose the ice cream has a smoother and more desirable melt-down. When the total solids are increased by increasing the serum solids and using 3% dehydrated culture as serum solids the stability decreases. If the total solids are increased by using a higher butterfat content and 3% dehydrated culture as serum solids, the stability of the ice cream increases.

M11 Utilization of Dehydrated Whey Solids in Ice Creams and Sherbets. J. H. GHOLSON, W. H. E. REID, R. J. BASNETT, AND R. M.

HANCKEL, Missouri Agricultural Experiment Station, Columbia.

Dehydrated spray process whey solids may be used in different flavored ice creams and sherbets without altering the processing, freezing or hardening procedure commonly applied in commercial ice cream plants. As much as 90% of the serum solids may consist of dehydrated spray process whey solids in the manufacture of ice creams and sherbets possessing a desirable flavor, body, texture and melt-down. Ninety per cent of serum solids in chocolate and strawberry ice creams also may consist of whey solids. Pineapple, orange and raspberry sherbets containing as much as 90% whey solids were very desirable in every respect.

The stability of flavored ice cream increases up to the point where 70% of the serum solids consisted of whey solids from spray process whey powder; 90% whey solids, tends to reduce stability again.

M12 The Relative Sweetness of Certain Corn Sweeteners in Ice Cream. L. D. HILKER, National Dairy Research Laboratories Inc.

Differences in the sweetness of the corn sweeteners used with sucrose in ice cream are recorded by a taste panel with the relative sweetness in descending order as follows: Cerelese, Sweetose, Hi-De and Frodex. Conditions which promote a high sweetness index of the corn sweeteners in ice cream are low percentage of corn sweetener, high ratio of sucrose to corn sweetener and high total sweetness. Selection of the panel, presentation to the panel of samples for comparison and evaluation of panel data are discussed. Tables suitable for use by ice cream manufacturers are given which show the amount of corn sweetener required to replace 15, 20 and 25% of the sweetness of ice cream having a total sweetness, as sucrose equivalent, of 15, 15.5, 16, 16.5 and 17%.

M13 The Sizes of the Colloidal Protein Particles of Skim Milk. T. F. FORD AND G. A. RAMSDALL, Bureau of Dairy Industry, USDA.

Sedimentation analyses of skim milk, made by using the laboratory centrifuge and the McBain air-driven ultracentrifuge, show that there are a limited number of different sizes of colloidal or acid-precipitable protein particles in skim milk. These sizes are definite, occur in two separate size ranges, and in each range the specific sizes are multiples of a unit. The particles are highly solvated, and the data indicate that all are spherical in shape. Although the specific sizes are definite, the distribution of sizes varies widely between milk samples, and is altered readily by

treatment. The particles in the two size ranges appear to represent two separate chemical entities, differing principally in phosphorus content. The diameter of the unit particle of the component present in the largest amount is about 640 Å. The gram particle weight of this solvated unit is about 94 million. The apparent molecular weight of the dry protein frame work is 33 million. The dominant particle in normal skim milk is composed of 16 of these units.

Calcium and casein nitrogen are removed together at the same rate as the colloids are removed from skim milk by centrifuging. This observation, combined with analyses of the separated colloids previously reported, leads to the conclusion that casein exists in milk as a calcium caseinate-calcium phosphate complex. If there is any concomitant free colloidal calcium phosphate, it is present in very small amount.

M14 Determination of Reducing Groups in Proteins and in Milk with O-iodosobenzoate.
B. LARSON AND R. JENNESS, Minnesota Agricultural Experiment Station.

A modification of Hellerman's (J. Am. Chem. Soc. 63: 2551. 1941) iodosobenzoate titration method for sulfhydryl groups in proteins has been developed. O-iodosobenzoate is added to a solution of the sample and after allowing 2 min. for reaction to occur, the solution is acidified and KI is added. The excess unreduced o-iodosobenzoate liberates iodine from the iodide and this can be titrated with thiosulfate. In Hellerman's original method starch indicator was used to detect the end point of the titration. This is subject to considerable error, particularly in titrating very dilute or opaque systems, because the end point is not sharp. Furthermore, proteins may adsorb or react with a considerable amount of the iodine liberated. In the present modification these difficulties are eliminated by an electrometric determination of the end point, which combines some of the features of "dead-stop" and amperometric titrations. The method has been applied to study of the sulfhydryl groups in proteins, including those of milk, and to determination of the effects of heat on some of the reducing substances of milk.

M15 Isolation of Minor Organic Compounds From Heated Milk. S. PATTON AND D. G. KEENEY, Pennsylvania State College.

Skim milk was heated to 122° C. for 60 min. Procedures used to remove, purify and concentrate minor organic substances included combinations of the following: addition of inorganic solute, solvent extraction steam distillation, and

normal and reduced pressure distillation. Data obtained concerning melting and boiling points, melting points of prepared derivatives, solubility characteristics, qualitative tests, etc., indicate the following types of compounds to be present in heated skim milk: (a) acids of the aliphatic series, such as acetic and butyric, (b) carbonyl acids, (c) sulfur-containing acids (nonamino), (d) mono and dicarbonyl compounds and (e) furan compounds. The specific identity of these components is being studied, the data being secured by the preparation of various crystalline derivatives.

M16 Milk Surfaces. II. Surface Tension Changes in Relation to Some Treatments of Milk. C. H. WHITNAH AND W. H. CHILSON, Kansas Agricultural Experiment Station, Manhattan.

A sample of freshly pasteurized milk was divided into three parts. Part 1 was not modified. Vitamin C was added to part 2. Copper sulfate was added to part 3. Immediately before studying the surface tension some of part 2 was homogenized in a hand homogenizer.

Surface tension then was measured on each of 4 samples at temperatures of 15, 20, 30 and 40° C. Another part of each sample then was diluted with water to 0.01% milk and to 0.001% milk. The time change of surface tension was measured at 25° C. These measurements were made after storage of 0, 2, 3, 4 and 8 days and on samples of fresh milk taken weekly beginning Feb. 7, 1949.

In all these samples of milk the temperature coefficient of surface tension was greater than previously reported for summer milk at Denver. Average temperature coefficient values were slightly less after addition of vitamin C and usually slightly more after addition of copper. These last two differences were of very doubtful significance.

For the diluted samples the differences in induction time before the first fall and in the rate of fall varied greatly. These variations are not related simply to the treatment of the milk nor to the age of the treated sample.

M17 Turbidity as a Means for Determining the Efficiency of Homogenization U. S. ASHWORTH, State College of Washington.

When 1 ml. of whole milk is treated with 5 ml. of 5N NH₄OH and diluted with 244 ml. water at 50 to 55° C., the resulting turbidity as measured 30 min. later in a photoelectric colorimeter is proportional to the size of the fat globules and the concentration of fat. When the fat content is known, the proportionality constant *K*, relating

to the size of the fat globules, can be calculated by dividing the photometric density by the final concentration of fat in mg./ml. This value for K has been shown to be proportional to the percentage of homogenized milk added to unhomogenized milk. When the Evelyn colorimeter is used to measure the turbidity, unhomogenized milk gives a K value of 1.1, while properly homogenized milk gives a value for K of 2.3. There is a close correlation between the homogenization pressure used and the turbidity, also between the U.S.P.H.S. cream rising index and the value for K .

M18 The Instability of Ascorbic Acid in Water, with Added Copper or Hydrogen Peroxide or Both.¹ R. W. BELL AND T. J. MUCHA, Bureau of Dairy Industry, USDA.

The effect of different factors, particularly the source of the water, on the stability of ascorbic acid solutions was investigated. Water may contain impurities that accelerate the oxidation of ascorbic acid and only pure water should be used in preparing an ascorbic acid solution that is to be used in standardizing 2,6-dichlorophenolindophenol with which the ascorbic acid content of milk and other fluids can be measured in acid solution by direct titration.

Since ascorbic acid is likely to be used in increasing amounts for retaining the fresh flavor of milk, the importance of correctly standardizing the dye solution used in measuring the ascorbic acid content of milk becomes apparent.

M19 Deferment of an Oxidized Flavor in Frozen Milk by Ascorbic Acid Fortification and by Hydrogen Peroxide Oxidation of the Ascorbic Acid of the Fresh Milk.¹ R. W. BELL AND T. J. MUCHA, Bureau of Dairy Industry, USDA.

Experiments were conducted to determine how long the onset of the characteristic oxidized flavor could be deferred in frozen storage by H_2O_2 oxidation of ascorbic acid in the fresh milk and whether fortification of the fresh milk with ascorbic acid would be as effective. Rapid and complete ascorbic acid oxidation in the fresh milk by H_2O_2 was effective in delaying but not in preventing the defect, since in all experiments the oxidized flavor was detectable eventually. The same was found to be true when the milk was fortified with ascorbic acid.

Somewhat better results were obtained when the fresh milk was fortified heavily with ascorbic

acid than when an optimum amount of H_2O_2 was added. Desirable properties of ascorbic acid for this purpose are mentioned and reasons for the stabilizing effect are described briefly.

M20 Electrometric Titration of Milk and Dairy Products in the Determination of Titratable Acidity. W. A. KRIENKE, Florida Agricultural Experiment Station.

A Model H Beckman glass electrode pH meter was used for "end point" determination in the electrometric titration of milk and various dairy products. The electrodes and a motor-driven glass stirrer were mounted in a holder to accommodate a 100-ml. beaker as the titration container.

With the stirrer in motion, 0.1 N alkali was added until pH 8.3 was reached, the amount of alkali required recorded, six drops of 1 per cent phenolphthalein solution added and titration completed to the "pink end point." By observing the movement of the pH meter needle, only 30 seconds are required to add the alkali to the sample. The "fading end point" and a change in the titratable acidity value due to cautious and slow titrations thus are eliminated.

Ranges in pH values obtained on the various products at the pink-color end point were for whole milk 8.53 to 8.65, condensed skim milk 9.00 to 9.08, cream 8.68 to 9.02, ice cream mix 8.70 to 9.02, skim milk 8.53 to 8.63, chocolate milk 9.10 to 9.30 and evaporated milk 8.96 to 9.04. The color of strawberry and cherry ice creams so obscured the phenolphthalein end point that it could not be established with certainty even at pH 9.5.

M21 A Re-evaluation of the Hortvet Formula and Freezing Point Value of Milk in Estimating the Percentage of Added Water. W. A. KRIENKE AND L. R. ARRINGTON, Florida Agricultural Experiment Station.

Samples of whole milk representing herds and individual cows of the Jersey and Holstein breeds were collected over a period of several months and their freezing points determined with a Hortvet cryoscope, using the recognized official method. Portions of some of the samples were diluted with known quantities of distilled water and their freezing points determined. Mean freezing point values were $-0.549^\circ C$. for 29 samples from individual Jerseys, $-0.539^\circ C$. for 13 samples from individual Holsteins, $-0.542^\circ C$. for 14 samples of herds of Jerseys, and $-0.535^\circ C$. for 6 samples of herds of Holsteins.

When the accepted value of $-0.550^\circ C$. is used in the formula for estimating the percentage of

¹ This work was done with funds from the Agricultural Research and Marketing Act of 1946.

added water, a false interpretation results. If the freezing point values for the diluted samples likewise are used, the calculated per cent of water added will be in excess of the amount known to have been added. Therefore it appears that a value somewhat higher than -0.550°C . must be established to permit better interpretations of water adulteration.

M22 Preliminary Observations of the Effects of Ladino Pasture and Hay Feeding on Tocopherol Content of the Fat and Stability of Milk. V. N. KRUKOVSKY, J. K. LOOSLI, AND D. A. THEOKAS, Cornell University.

Observations indicated that the levels of tocopherols in the milk fat are influenced by the type and qualities of the roughages fed to the cow and that the relationship might exist between the tocopherol content of the fat and the susceptibility of milk to oxidized flavors. Feeding ladino pasture resulted in tocopherol levels of the fat considerably higher than were found when ladino hay was included in the ration, but not as high as when the cows were transferred to birdsfoot pasture. A gradual increase was observed in the tocopherol content of the fat from 2,938 γ (av. value) per 100 g. of fat at the end of ladino pasture to a plateau level of 4,350 γ during the third and fourth weeks of birdsfoot pasture. Milk of poor keeping quality resulted during the ladino feeding (both pasture and hay), whereas the transfer of the cows from ladino to birdsfoot pasture resulted in an appreciable stabilization of milk, even in the presence of added copper. The transfer of the cows from birdsfoot pasture to hay feeding in the barn resulted in the decrease in the tocopherol content of the fat and greater susceptibility of milk to oxidized flavors depending upon the kind and quality of hay fed.

M23 Effects of External Temperature and Pasturage on the Degree of Unsaturation of Milk Fat. E. E. BARTLEY, E. W. BRID, C. Y. CANNON AND J. H. ZALETEL, Iowa State College.

The objective of this study was to separate the effects of seasonal changes in temperature from the effects of pasture feeding to determine the relative importance of these two factors in producing the highly unsaturated milk fats encountered during the summer. Twelve Holstein cows freshening at various times during the experiment (duration 13 months) were divided into two similar groups. Both groups at first received prairie hay and a grain mixture. One of the groups continued on this ration for the entire trial

while the other group was placed on pasture during the grazing season, pasture grass replacing the hay. The cows receiving grass grazed only at night, while the other cows were placed in a dry-lot where they received hay. Daily records of the environmental temperatures were kept. Iodine and thiocyanogen values were determined on weekly samples of butterfat from each individual animal.

Pasture and stage of lactation have primary influences on the degree of unsaturation of milk fat, while changes in temperature appear to be of considerably less importance. Production of highly unsaturated fats at the peak of milk production, followed by a gradual decrease in unsaturation to about the fifth month and then a gradual increase continuing to the end of lactation, appear to be definite lactation trends.

M24 Separation of Fatty Acids by Displacement Chromatography and its Application to Analysis of Butter Fat. R. T. HOLMAN AND L. HAEDAHL, A. AND M. College of Texas.

Using the displacement development technic and the absorption analysis apparatus of Tiselius, fatty acids from C_1 to C_{22} have been separated. By using fatty acids as displacers and by choosing suitable solvent conditions, homologs of 1 carbon atom difference can be separated and measured. Unsaturated acids can be separated from saturated acids. Usual sample sizes of 50 to 200 mg. can be separated into the component acids with 90 to 100% recovery in chromatograms developed in 3 to 6 hr. Typical experiments were described and results shown. Application to analysis of dairy products were discussed.

M25 Some Observations on Fat Fractions from Butter Oil. A. T. MUSSETT, S. PATTON AND C. D. DAHLE, Pennsylvania State College.

Liquid and solid fat fractions were obtained by hydraulic separation of fresh butter oil using a Carver laboratory press. Separation at 70°F . gave approximately 30% solid and 70% liquid; at 50°F ., 80% solid and 20% liquid; at 32°F ., 90% solid and 10% liquid. The acid and iodine numbers of the 70 and 50°F . solid fractions were significantly lower than those of the corresponding liquids. The keeping qualities of the solid fats, as determined by organoleptic tests, were much superior to those of the liquid samples. A preliminary trial using the 70°F . solid fraction as the sole source of fat in dry whole milk has produced a product with improved flavor. The high melting fractions should constitute a suitable form in which to store fat for ice cream.

M26 The Steam Distillation of Stale-flavor Component from Stale Butteroil. R. McL. WHITNEY, KATHERINE PAULSON AND P. H. TRACY, University of Illinois.

A procedure was developed which was successful in distilling the stale-flavor component along with other volatile components from stale butteroil. The petroleum ether-soluble portion of this volatile fraction was found to contain approximately 10,000 times as much stale-flavor component per unit weight as the dried whole milk from which it was obtained originally. This procedure involves the steam distillation of stale butteroil under a pressure of approximately 70 mm. of mercury and at a temperature of approximately 45° C. The distillate is collected in water at 0° C. and, after distillation, this aqueous suspension is extracted with petroleum ether. The fraction containing the stale-flavor component then is obtained by removing the petroleum ether by aspiration.

It also was observed that the stale-flavor component apparently is synthesized in the stale butteroil during steam distillation, since the concentration of the stale-flavor component in the residual butteroil after distillation was the same as or greater than that in the original butteroil.

M27 The Extraction of Stale Butteroil from Stale Dried Whole Milk by Organic Solvents. R. McL. WHITNEY AND P. H. TRACY, University of Illinois.

In a search for a more efficient method for obtaining stale butteroil from stale dried milk, several different Soxhlet type extractions were investigated with variations in the type of powder, pretreatment of the powder and extracting solvent.

Spray-drying uncondensed, unhomogenized milk at low pressures yields a product from which much higher recoveries of butteroil are obtained than from a milk powder prepared in a similar manner from a condensed milk.

Hydration to 8% moisture of a dried milk prepared at low spray pressures from condensed unhomogenized milk results, upon extraction, in a high yield of butteroil. If a milk powder prepared in this manner is agitated with 95% alcohol plus sufficient water to hydrate the powder to 8% moisture, high recoveries of butteroil are obtained, provided the fat dissolved in the alcohol is recovered. Anhydrous ethyl ether and petroleum ether are suitable solvents.

Upon the development of a special solvent-removal technic to reduce the solvent concentration so as not to interfere with organoleptic tests, the stale-flavor component was found to be extracted

with the butteroil in the same proportion as it occurs in the fat in the dried whole milk.

M28 Sanitary Standardization of Equipment Used in the Dairy Industry. E. H. PARFITT, Evaporated Milk Assoc., Chicago.

To secure uniformity of design and to reduce the cost of equipment the dairy industry has created a committee known as the Sanitary Standards Committee. This committee represents the seven branches of the dairy industry. Equipment common to all branches of the industry is being reviewed for the purpose of writing sanitary standards that will meet the approval of the International Association of Milk and Food Sanitarians and the U. S. Public Health Service. On approval by these two latter organizations, these standards are published and companies fabricating equipment meeting these standards designate their equipment as 3-A.

Standards for finish of stainless steel, minimum knuckle radius, minimum slope for drainage, maximum size for ease of cleaning, ease of disassembly, etc., have been developed for pipe line fittings, milk storage tanks, weigh cans and receiving tanks, and homogenizers.

Tentative standards are now in the hands of the industry and regulatory groups for approval of the following pieces of equipment: milk transportation tanks, heat exchangers (plate, surface and tube), milk filters, milking machines, milk pails and strainers, can washers, electric motors and, for point-of-sale equipment, ice cream cabinets and soda fountains.

M29 Nutrition of the Lactic Group of Streptococci and its Relation to Bacteriophage Multiplication. E. B. COLLINS, F. E. NELSON AND C. E. PARMELEE, Iowa Agricultural Experiment Station.

Sodium acetate and/or Tween 80 were found essential, as shown by turbidity measurements, for the growth of 9 of 32 strains of *Streptococcus lactis* and 22 strains of *Streptococcus cremoris* in a chemically defined medium based upon that of Niven (J. Bact., 47: 343-350, 1944). Reticulogen, a commercial liver extract, could be substituted in somewhat smaller quantities for sodium acetate and Tween 80 and also was the only supplement which permitted rapid growth of one strain of *S. cremoris*, which did not become turbid until after 24 hr. in the medium supplemented with sodium acetate and Tween 80. Neither sodium acetate nor Tween 80 alone would suffice for serial transfer of most cultures; however, sodium acetate alone was effective for more cultures than was Tween 80 alone. Most strains

of the lactic group which had been in the laboratory for considerable time were found to be *S. cremoris*, while all recently isolated strains were found to be *S. lactis*, on the basis of ammonia formation from arginine and growth at 40° C.

With two *S. lactis*-bacteriophage combinations, multiplication of both bacteriophage and organism were affected similarly by the omission of individual components from the unsupplemented complete medium of Niven. Bacteriophage multiplication seems to be associated closely with organism multiplication for these two combinations. However, with a third *S. lactis*-bacteriophage combination, bacteriophage multiplication does not occur in the defined medium which is adequate for organism growth.

Further studies of the relationship between organism multiplication and bacteriophage multiplication in defined media are being made.

M30 Thermal Death Time Studies of Coliform Bacteria in Milk. J. C. OLSON, JR., H. MACY AND H. O. HALVORSON, University of Minnesota.

Each of 139 coliform cultures was assayed for heat resistance at 135° F. A multiple-tube technique similar to that used by Slatter and Halvorson (J. Dairy Sci., 30: 231-243. 1947) was used. Thermal death times at 135° F. varied from less than 5 min. to over 150 min. The most heat-resistant cultures were, almost exclusively, members of the *Escherichia coli* section as described by Parr (Bact. Rev., 3: 1-48. 1939). On repeated trials under similar conditions of culture manipulation, thermal death time variations of a pure culture of *E. coli* ranged from 39 to 74 minutes at 140° F. Similar variations occurred at other temperatures.

In two trials the *z* values (Bull. Nat. Research Council. U. S., 7: Part I, no. 37. 1923) of thermal death time curves for suspensions of *E. coli* (50,000 cells/ml.) in milk, prepared from cultures grown in milk at 37° C. for 16 hr., were 10.5 and 10.8. For suspensions prepared from cultures grown at 37° C. for 24 hr., *z* values of 10.0 and 11.0 were obtained. The *z* values of thermal death time curves for similar suspensions of *E. coli* prepared from cultures grown at 20, 30 and 37° C., each at a comparable stage of growth, were 9.7, 11.4 and 11.5, respectively. With suspensions containing 5 million cells/ml., prepared from cultures grown at 37° C. for 24 hr., *z* values of 11.7 and 13.0 were obtained.

The slope of a curve plotted on semi-logarithmic paper by connecting with a straight line two points, one representing low-temperature, holding (143° F. for 30 min.) and the other representing high-temperature, short-time pas-

teurization (160° F. for 15 sec.), may be expressed by a *z* value of 8.25. Significantly all *z* values of thermal death time curves obtained were greater by a considerable margin than the *z* values of the "pasteurization curve". The data obtained in this regard provide evidence in support of the theoretical explanation advanced by Ball (Ind. Eng. Chem., 35: 71-84. 1943) for the observed differences in bacterial destruction by the two processes.

M31 Studies on Acid Production, Loss of Bacteriophage and Resistance of a Bacteriophage-sensitive Culture of *Streptococcus lactis*. H. F. FORD AND F. J. BABEL, Purdue University.

The influence of bacteriophage on the acid-producing ability of a culture of *Streptococcus lactis* and the duration of bacteriophage in a sensitive culture were studied. The acid-producing ability of the culture studied was influenced greatly by an initial inoculation of bacteriophage; however, after secondary growth occurred, future transfers of the culture were approximately as active as the same culture maintained in a bacteriophage-free condition, although the one culture carried bacteriophage. Some of the cultures given an initial inoculation of bacteriophage and carried through several transfers finally became free of bacteriophage. The loss of bacteriophage did not influence the rate of acid production of the culture.

The time required for a sensitive culture to become free of bacteriophage when given an inoculation of bacteriophage was dependent upon the temperature of incubation. A culture inoculated with bacteriophage and carried at a temperature of 37° C. became free of bacteriophage after 11 transfers. The same initial culture inoculated with bacteriophage and incubated at 26° C. for 10 propagations and further incubated at 21° C. lost bacteriophage after 35 transfers. Also, the same initial culture inoculated with bacteriophage and incubated at 21° C. was not free of bacteriophage after 127 transfers.

A culture inoculated with bacteriophage and transferred until the culture was bacteriophage-free was not sensitive to the bacteriophage with which it was treated but was sensitive to other bacteriophage preparations.

M32 Variations Encountered in the Grading of Raw Milk with the Methylene Blue and Resazurin Reduction Tests. R. K. LEWTON, D. M. MARKLAND AND F. J. BABEL, Purdue University.

Excellent correlation was obtained between the methylene blue reduction test and the resazurin

reduction test when both tests were read at the time the dyes were reduced completely. However, when comparisons were made on the grading of raw milk by the methylene blue test and the triple-reading resazurin test read at the pink end point (Munsell Standard P 7/4), the resazurin test placed considerably more samples in the poorer classes than the methylene blue test. Variations in the results obtained could be explained partially on the basis of the body-cell count.

The plate count and direct microscopic count classified 66% of the samples alike and there was a difference of one grade with 29% of the samples. The methylene blue test classified 72% of the samples the same as the plate count and there was a difference of one grade with 24% of the samples. The resazurin triple-reading test classified 41% of the samples the same as the plate count, 20% in a classification one grade lower, 18% two grades lower and 17% three grades lower than the plate count. Approximately 4% of the samples were graded higher by the resazurin test.

Most of the samples of raw milk showing extreme differences in classification, when using the plate count and resazurin triple-reading test for grading, had plate counts of 50,000 or less.

M33 Standards for Grades of Milk for Use in Manufactured Dairy Products. C. J. BABCOCK AND H. J. EMERY, Manufactured Dairy Products Division, Dairy Branch, PMA, USDA.

One of the main obstacles to quality improvement is the lack of uniform and generally recognized standards of grades for milk and cream. Such standards would provide a uniform basis for buying milk and cream according to quality and for otherwise carrying out quality improvement work. The U. S. Department of Agriculture, in cooperation with dairy industry organizations and state agencies, therefore is formulating standards for milk and cream grades. The response to the tentative draft of these standards indicates a need for such standards. A summary of the comments received from industry organizations and State agencies will be presented.

M34 The Effect of Certain Metallic Ions on the Germicidal Activity of Quaternary Ammonium Germicides. W. S. MUELLER AND D. B. SEELEY, University of Massachusetts.

A study has been made on the effect of metallic ions on the germicidal action of quaternary ammonium compounds. Elements were selected from the periodic table in such a manner as to

compare light and heavy metals, metals of different atomic weights and metals of different valences. Salts that contained an anion which showed no measureable interference were used so that the effect of the cation alone could be studied. The study also involved an understanding of the effect of pH on germicidal action since the pH values of the salt solutions varied.

Valence and pH were the two most important factors determining the interfering power of a cation. Monovalent, divalent and trivalent cations had interfering power approximately in the ratio of 1:100:10,000, respectively. Trivalent ions frequently inactivated the quaternary 100% in concentrations as low as 10 p.p.m. Five times as much quaternary was required for 100% kill at pH 3 than at pH 10. When adjusted to pH 7, the cations lost their interfering power. Atomic weight had little or no effect on inactivating power. The salts seem to interfere by competing for the cell surface and thus blocking the germicide cation.

M35 Optimum Consumer Preference for Dry Milk in Bread. E. L. JACK AND (MRS.) V. M. HAYNES, University of California.

A group of 320 boys ranging in age from 8 to 16 years in a self-contained unit of the California Youth Authority have been fed breads containing different quantities of non-fat dry milk solids as part of their regular diet. The percentages chosen were 0, 6, 10 and 14% non-fat dry milk solids, based on the amount of flour. These breads each were fed for a 2-mo. period during which accurate bread consumption and total food consumption records were kept. Aliquot samples of each meal were composited for chemical analyses and height and weight records were kept at the beginning and end of each period.

The data show that the diets were superior to the recommended allowances of the National Research Council in caloric, protein, vitamin and mineral values. The bread consumption averaged for each 2-mo. period was 100% for the water bread, 103.8% for 6% bread, 105.6% for the 10% bread, and 114.5% for the 14% bread. The breads were essentially uniform in appearance.

M36 The Utilization of Roller and Spray Dried Sweet Cream Buttermilk in Bread Making. J. V. REGER, W. B. COMBS, S. T. COULTER AND R. B. KOCH, University of Minnesota.

Dry sweet cream buttermilk and nonfat dry milk solids obtained from the same source of mixed herd milk were adjusted to various moisture levels and stored at 100° F. and 42 to 45° F.

for 1 yr. Baking tests showed that prolonged storage at 100° F. was detrimental to the baking quality of the milk powders, especially with the roller dried samples of high moisture content (5-6%). No differences were noted in the baking quality of milk powders stored for 1 yr. at 42-45° F. Breads made utilizing the buttermilks, particularly spray dried, gave consistently larger loaf volumes than when nonfat dry milk solids were used.

Additional heat treatment of buttermilk, which was obtained from sweet cream pasteurized for 30 min. at 165° F., did not affect the baking quality of the dried product. Buttermilk powders again were associated with larger loaf volumes as compared with nonfat dry milk solids.

The addition of 0.17% soybean lecithin emulsified in butter oil and added to skimmilk before drying produced the same loaf volumes when added to the bread formula as did the buttermilk powders. Additions of butter oil alone to skimmilk showed no beneficial results with respect to loaf volume.

A lactic acid culture (3%) was added to sweet cream buttermilk and when 0.01, 0.05 and 0.1% developed acidities were obtained, the buttermilks were neutralized to the original acidity. Resulting powders, when added at a 6% level to the bread formula, produced no detrimental effects which could be attributed to the amount or to the type (NaOH or Lime) of neutralizer, even after storage for 2 mo. at 100° F.

M37 The Relation of Surface Growth to the Ripening of Minnesota Blue Cheese. H. A. MORRIS, W. B. COMBS, S. T. COULTER, University of Minnesota.

Green cheese from each of two lots were segregated into three groups and processed to produce various amounts of slime during the ripening period. The cheese were examined chemically and organoleptically initially and at 3, 6 and 9 mo. of age. In addition, portions of the cheese were analyzed to determine whether ripening had proceeded inward from the exterior of the cheese. Moisture, sodium chloride, total volatile acidity, total nitrogen, amino nitrogen, pH and fat content of the cheese were determined. The microscopic and macroscopic characteristics of the surface microflora were observed.

The initial development of yeasts and some mold on the surface of the Blue cheese was followed by a predominance of rod forms of bacteria and some micrococci at later stages.

Because of the pronounced effect of *P. roqueforti* on the ripening of this cheese, it is difficult to determine the influence of the surface microflora on the changes noted in the various portions

of the cheese. However, cheese with normal surface growth had a higher total volatile acidity, amino nitrogen and pH, a finer flavor, and a more desirable texture than cheese with no surface growth. Cheese slimed for 4 mo. had excellent body and texture, but also had an undesirable limburger-like flavor due to the absorption of aroma from the slime. These cheese had the highest total volatile acidity, amino nitrogen and pH.

M38 The manufacture of Blue Cheese from Pasteurized Homogenized Milk. I. I. PETERS AND F. E. NELSON, Iowa Agricultural Experiment Station.

A study was made of the separate influences of: (a) heat treatment of milk before and during homogenization, (b) setting acidity, (c) dipping acidity, (d) cooking temperature, (e) dipping temperature, (f) early salting and (g) draining temperature upon texture, mold growth, body and flavor of ripening blue cheese.

As a result of these studies, the following make procedure was adopted: Fresh, raw milk was pasteurized at 142° F. for 30 min., cooled to 110° F. and homogenized at 2,000 lb. pressure. The milk was set at 90° F. and ripened with 1% lactic cheese starter to 0.19% acidity. Lipase from *Candida lipolytica* was added at a rate to give the desired degree of ripening and the milk set with 90 ml. rennet per 1,000 lb. of milk and allowed to set for 70 min. The curd was cut into 0.5 in. cubes and held in the whey, being stirred at 30-min. intervals, until the whey acidity reached 0.24%. Part of the whey was drawn off, leaving only enough in the vat to cover the curd, and the curd cooled to 84° F. with cold water in the outer jacket. The remaining whey was drawn off, 0.01% mold powder mixed with 1.5% cheese salt, based on the calculated yield of cheese, incorporated into the curd, and the curd hooped. The cheese was drained overnight at 84 to 86° F., dry-salted, punched and cured in the usual manner.

This procedure permitted manufacture from pasteurized, homogenized milk of a cheese with sufficiently open texture, abundant mold growth, good body and adequate flavor.

M39 The Determination of Free Tryptophane in Cheese. A. B. EREKSON, Lakeshire-Marty Company, Plymouth, Wis.

A test for free tryptophane based upon the one reported by Dugan (J. Assoc. of Official Agr. Chemists, 31, 1. 1948) was developed using a 2-g. sample of cheese, 90% acetone to extract the tryptophane and to precipitate the proteins, para-dimethylaminobenzaldehyde as the color

reagent and KNO_2 as the oxidizing agent to hasten the reaction. Quantitative results were obtained by comparison with a standard graph from readings with an electrophotometer.

Cheese with a mild flavor had from 15 to 91 γ of tryptophane/g. while cheese high in flavor contained up to 889 γ /g. Experimental cheddar cheese made from raw and pasteurized milk and cured for 9 mo. at 55 to 60° F. showed an average of 297.3 γ for the raw milk cheese and only 117.3 for the pasteurized milk cheese. The raw milk cheese was much higher in flavor than the latter.

M40 Filter Paper Chromatography as a Means to Determine the Amino Acids and Amines Developed in Cheddar Cheese During Ripening. F. V. KOSIKOWSKY, Cornell University.

Two-dimensional filter paper chromatography was applied to the water extracts of a number of Cheddar cheeses. Solvents used were phenol and a mixture of collidine and lutidine. A butyl alcohol solution of ninhydrin was employed as a color developer.

Using this method a large number of free amino acids from individual cheeses simultaneously were pictured as colored areas on Whatman No. 1 filter paper sheets. Also observed on the same chromatograms were amines and, in some instances, a number of unidentified chemical compounds.

In one series of eight commercial Cheddar cheese analyzed, the following amino acids and amines were interpreted from the chromatograms as being present in most of these cheeses: leucine, valine, α -amino-n-butyric acid, alanine, threonine, glycine, asparagine, glutamic acid, aspartic acid, arginine, lysine, proline, tyrosine and glutamine. Methionine as methionine sulphoxide tentatively was identified as being present in a smaller number of the cheeses. The presence of tyramine and tryptophane also was considered in some of the cheeses, but their spots are more difficult to identify. Other free amino acids may have been present in these Cheddar cheeses, but additional technics may be required to reproduce them on chromatograms.

M41 Manufacture of Cottage Cheese from Reconstituted Non-Fat Dry Milk Solids. C. E. PARMELEE AND W. S. ROSENBERGER, Iowa Agr. Expt. Station.

A method for the manufacture of cottage cheese from non-fat dry milk solids is presented. The method has been used successfully with commercial low heat treatment non-fat dry milk solids from three sources. The method is presented as a means of holding cottage cheese markets during periods of extreme shortage of fluid skim milk, as the cost does not permit competition with cottage cheese from fluid skim milk when prices are normal.

PRODUCTION SECTION

P1 Differences in Production Type, Size and Breeding Efficiency of Cow Families. K. A. TABLER, W. J. TYLER AND G. HYATT, JR., West Virginia University, Morgantown.

Nineteen cow families in the Reymann Memorial Ayrshire Herd at the West Virginia Agr. Expt. Station were studied to determine if there were differences between families in production, type, body size and breeding efficiency. In addition, family selection was compared with selection on individual performance as a means of obtaining improvement in the milk production of the herd. The data consisted of the individual milk, butterfat, butterfat test, type, body size (height at withers and weight) and breeding records collected between 1922 and 1948 on 401 cows. All normal females were kept until they completed at least one lactation.

Analyses of variance showed that differences between families in body size and breeding efficiency were not significant. However, there were highly significant differences between families for milk, butterfat, butterfat tests and type. When sire differences were eliminated, the remaining

variations between families were not statistically significant.

Two methods of selection based on the first record were compared. If each year (beginning in 1928) two-thirds of the first calf heifers with the highest milk production had been retained and the lowest third culled, the offspring of the selected parents during the next 10 yr. would have averaged 8,625 lb. of milk and 10,434 lb. during the following 10 yr. But if the cows and their offspring in the 12 families (two-thirds of all families) with the highest average milk production in 1928 had been kept, the averages of their daughters during the first and second 10 yr. periods would have been 7,759 and 9,767 lb. of milk, respectively.

P2 Prolonged Gestation of Genetic Origin in Cattle. S. W. MEAD, P. W. GREGORY, AND W. M. REGAN, University of California, Davis.

Twenty-seven cases of prolonged gestation (310 to 350 days) have been observed in a large herd of registered Holsteins. Birthweights of calves

have ranged from 110 to 168 lb., averaging 145 lb.

With the exception of two lighter weight calves, normal delivery has been possible. Four have been delivered by Caesarean section. Of these none has survived more than a few hours. All other calves were dismembered. As a result, the future reproductive capacity of these cows has been impaired greatly. During the ninth month of pregnancy, there appears to be a complete absence of all physical changes normally occurring.

Pedigree analyses indicate that all calves manifesting this anomaly are homozygous for an autosomal recessive gene. It is concluded tentatively that prolonged gestation is caused by an hormonal imbalance between the fetus and mother, when the fetus is of the mutant genotype. This unique genetic material should prove valuable for certain physiological studies.

P3 Estimation of Changes in Herd Environment. C. R. HENDERSON, Cornell University, Ithaca, N. Y.

Accurate appraisals of the results of breeding programs and most efficient estimates of breeding values of individuals whose own records and whose relatives' records were made in several different years require quantitative measures of the effects of changing herd environment. Least squares or modified least squares methods for obtaining such measures give biased estimates when records of cows culled from the herd are either above or below the herd average. This bias results from the lack of perfect repeatability of records. In contrast, the method of maximum likelihood automatically takes into account incomplete repeatability and annual culling levels and utilizes all of the records in such a way as to obtain the most precise estimates possible of the yearly environmental effects.

The maximum likelihood method has been utilized to obtain annual correction factors for several New York dairy herds. The method is illustrated with data from one of these herds and less accurate but less laborious modifications are described. Examples are given of the use of the correction factors for estimating the genetic improvement in the herd, predicting the breeding values of cows and evaluating sire proofs. Application of the method to computation of age correction factors also is discussed.

P4 The Number of Proved Sons Necessary to Evaluate the Transmitting Ability of a Sire. W. E. WASHBON and W. J. TYLER, West Virginia University, Morgantown.

One hundred seventy-four Holstein sires with eight or more D.H.I.A. proved sons were studied to determine the least number of proved sons necessary to estimate most accurately the performance of those to be proved later. The data included average butterfat production of the proved sons' daughters, average difference of daughters' production as compared to their dams and per cent of proved sons that maintained or increased butterfat production in the herds in which they were used. Averages of the first three to ten proved sons, respectively, were compared with averages of the following three, five and ten sons.

Highly significant correlations ($r = 0.35$ to 0.65) indicated that the average butterfat production of the daughters of the first three proved sons was nearly as accurate as data on more sons in estimating the average butterfat production of the daughters of the next three, five or ten proved sons of a sire.

Similarly, the significant correlations ($r = 0.24$ to 0.40) for the sons' daughters' increase or decrease in butterfat production from their dams indicated that data on the first three or four proved sons were nearly as accurate as data on a larger number in predicting what might be expected from the next three, five or ten proved sons in this respect.

For per cent of sons improving production the correlations were significant when the first four, five and six sons were compared with the next ten sons ($r = 0.30$).

A sire's future granddaughters' butterfat production and its difference from their dams' production apparently can be estimated nearly as well from the performance of the first three or four sons as from a larger number. The per cent of a sire's future proved sons that likely will improve production is more reliable if the prediction is based upon the performance of at least the first four or five proved sons.

P5 Calf Mortality, Sex Ratio and Incidence of Twinning in Two University of Minnesota Herds. K. MILLER and L. GILMORE, Minn. Agr. Expt. Station, St. Paul.

The University calf records at Grand Rapids and St. Paul have been analyzed with respect to prenatal and postnatal mortality for the first 6 mo. and for other information such as the sex ratio and incidence of multiple births.

At University Farm for the 12 years 1934 to 1945, inclusive, 50 of the 592 births, among Guernseys, Holstein-Friesians and Jerseys, were born dead. Ninety-one died during the first month, 36 more by the end of the sixth month

and 391 were in the herd at the end of 6 months of age. The 24 remaining calves were mostly bulls that were destroyed, sold for breeding purposes or utilized in an experiment, and are excluded from the above groups. The principal causes of death were pneumonia and scours, although some genes for lethals or lack of vigor appear to have some influence.

In the Grand Rapids herd during the 36 years 1912 to 1947, inclusive, 1,007 high grade and purebred Guernsey calves were born. Of these, 61 were born dead, 61 died during the first month and 50 from 2 to 6 months. Twenty-two deaths resulted from scours and 15 from pneumonia.

The sex ratio of 52 per cent for 1,143 Guerneys from both herds is higher than that previously reported for this breed and is close to the average for all cattle reported. For 229 Holstein-Friesians the ratio was 52%. For 159 Jerseys the ratio of 46% is lower than that reported elsewhere for this breed.

Twinning occurred in 1% of the 996 Guernsey births in the Grand Rapids herd as compared to 3% of 169 births in the St. Paul herd. For Holsteins and Jerseys the incidence was 5 and 1%, respectively. In 30 pairs of twins there were 19 bisexed pairs. No triplets were born in over 1,165 births.

P6 Observations on Mammary Gland Development of Dairy Heifers Induced by Hormone Injections.¹ J. F. SYKES, T. R. WRENN, AND P. C. UNDERWOOD, Bur. of Dairy Industry, USDA, Washington D. C.

Two heifers were injected with stilbestrol, three with stilbestrol plus progesterone, two with stilbestrol plus a crude pituitary extract and two with stilbestrol plus progesterone plus a crude pituitary extract. Injections were started at 1 mo. of age and continued to 9 mo. Examinations of mammary tissue were made at 5 mo. of age and at autopsy (9 mo. of age). No visible increase in the size of the mammary glands was produced by these treatments. As judged by palpation, the glands of the experimental heifers appeared to be slightly smaller than those of two control heifers. A small amount of secretion was obtained from all the experimental heifers but was not sufficiently copious to make comparisons possible.

Histological examination of mammary glands of all injected heifers showed marked glandular development, with evidence that this development was not altogether normal. At 5 mo. of age only those heifers which received steroid hormones showed lobule-alveolar development, the extent

of this development being unaffected by progesterone. At 9 mo. of age, however, all heifers showed lobule-alveolar development and in general the heifers which received pituitary hormones in addition to the steroid hormones were further advanced than heifers receiving only steroid hormones. Again progesterone did not appear to influence the extent of development.

Weights of pituitary, thyroid and adrenal glands, and of the ovaries and bioassays of the pituitary gland indicated that the steroid hormones produced at least a partial functional hypophysectomy which was counteracted to some extent by the injection of the pituitary preparation used.

P7 Effect of Temperature and Drying on Male Hormone in Cow Manure. C. W. TURNER, Mo. Agr. Expt. Station, Columbia.

In previous studies of the male hormone content of dairy cow manure, the fresh product was dried in a Freas electric oven at a temperature of 45° C. This drying temperature was selected since it had been shown that with increasing temperature up to 85° C., the biological activity of the male hormone present was inactivated when assayed by feeding to chicks for a period of 4 wk. It seemed of interest to determine the biological activity of the fresh product and of manure dried by other methods.

Surprisingly, the feeding of amounts of fresh manure equivalent to 10% of dry manure was without biological activity. Further, when the cow manure was heated for varying periods of time at 45° C. without drying, biological activity was not demonstrated. It thus appeared that drying, rather than heat, plays a role in converting biologically inactive male hormone to an active form. An alternative suggestion is that the enzymes and bacteria present in fresh manure may inactivate the hormone before it is consumed. A number of experiments designed to obtain answers to these questions will be presented.

P8 Effect of Mild Hyperthyroidism on Milk Production in Dairy Cattle. C. W. TURNER, Mo. Agr. Expt. Station, Columbia.

The inheritance of the capacity for high milk production depends upon the rate of secretion of the hormones which influence the growth of the secretory tissue and the initiation and maintenance of milk secretion. It has been demonstrated that the thyroid hormone when available in insufficient amounts depresses milk secretion and by mild replacement therapy stimulates milk secretion up to the point where some other hormone limits production.

¹ This work was done with Bankhead-Jones special research funds.

It seems reasonable to believe that in the selection of cows for high milk and fat production that high thyroid secretion rate is essential and when combined with high secretion rate of other hormones outstanding cows are bred. However, since selection at this time is not based upon the secretion rate of the various hormones, in many cases cows are bred with an inheritance for milk production considerably above their actual production simply because of a deficiency in the cow's thyroid hormone secretion rate. By the simple process of feeding thyroprotein, this deficiency is overcome and the full inherited potentialities of the cow are then realized. It should be emphasized that the feeding of thyroid hormone has exactly the same effect upon the cow as the secretion of optimum amounts of hormone by the animal's own thyroid.

The question is being raised as to the physiological effect of a mild hyperthyroid condition occurring either through inheritance or induced by thyroprotein feeding. The effect on feed consumption, energy metabolism, heart rate, respiration rate, body temperature and longevity will be discussed.

P9 Effects and Economy under Tennessee Conditions of Thyroprotein Feeding during Lactation Decline. E. W. SWANSON, University of Tennessee, Knoxville.

Twelve cows were paired carefully according to production, size, age, and time of parturition and were assigned randomly one of a pair to a control group and the other to a thyroprotein-fed group. The cows were fed to try to maintain desirable production and body condition. Following evidence of a definite downward trend in the lactation curve (average of 122 d.), thyroprotein was fed at the rate of 15 g. per day. Small responses in milk production but very little change in fat test occurred. Slight temporary increases in pulse, body temperature and respiration occurred following the thyroprotein feeding. These values returned to normal after 8 w. of thyroprotein feeding, and at this time the extra milk production stimulus also had ceased. During this time the thyroprotein-fed cows lost slightly in body weight, while the control cows gained. Thyroprotein feeding plus more concentrates was continued until the end of the lactation. The thyroprotein-fed cows again were normal in weight at the ninth lactation month. For an average of 183 d., thyroprotein feeding resulted in 0.76 lb. more milk produced and 0.54 lb. more concentrates consumed per cow daily. The same cows are being continued for a second lactation to measure the carry-over and/or repeatability of the effects.

P10 Size of Thyroid in Cows from Southern States. W. W. SWETT AND C. A. MATTHEWS, Bureau of Dairy Industry, USDA.

At most of the field stations operated by the Bureau of Dairy Industry in the southern states, many individual cows have failed to produce satisfactorily and the production level of one herd has declined despite the fact that sires known to possess good transmitting ability have been used and environmental conditions have been maintained on a reasonably uniform basis.

A number of cows with particularly low producing records, from families of high producers, were shipped to Beltsville for slaughter. In two average-sized Guernseys from the station at Columbia, S. C., the weight of thyroid was found to be only 56% of the average for Guernseys obtained in the Bureau's anatomical studies. In seven Jersey cows shipped to Beltsville from the station at Jeanerette, La., the weights of thyroid were only 56% of the average found in Jerseys only slightly larger in the Beltsville herd. Also, in 14 other Jerseys from the Jeanerette herd that were slaughtered in La., very low thyroid weights were found. Anatomical studies of cows from various areas showed a definite tendency for the thyroids of cows in the southern states to be much smaller than the thyroids of cows of the same breed in northern states.

P11 Factors Affecting Heart Rates of Dairy Cows. J. W. THOMAS, Bureau of Dairy Industry, USDA.

Heart rates were observed on approximately 40 cows for varying periods of time up to three lactations. All observations were made under standardized conditions in order to eliminate factors that are known to affect heart rate.

The heart rate of producing cows increased when they consumed T.D.N. at 120 to 130% of requirement. The maximum increase in heart rate was not attained until 30 to 40 d. after the increase in T.D.N. consumption was initiated.

Feeding thyroprotein also increased the heart rate of producing cows. An additional effect on heart rate was noted when thyroprotein and extra T.D.N. were fed simultaneously. Thyroprotein produced only a transient increase in heart rate when T.D.N. was consumed at 100% of requirement.

Heart rate was faster in early lactation than during the second half of the lactation period. A rapid decrease in heart rate usually was accompanied by a rapid decrease in milk production. Most cows show a definite increase in heart rate immediately after they have been dried off. This increase is not a result of the pre-

calving increase. Heart rate increased rapidly in the dry cow just before calving. At 70 to 90 d. before calving it averaged 65 beats/min., at 30 to 50 d. it averaged 72, and at 0 to 10 d. it averaged 92.

A cow usually had a more rapid heart rate during estrus than immediately before or after estrus.

P12 Milk Substitutes for Young Dairy Calves.

H. D. WALLACE, J. K. LOOSLI AND K. L. TURK, Cornell University.

Nine milk-substitute formulas have been studied in preliminary trials using 75 male and female Holstein calves. The Cornell dry calf starter method of feeding was followed, except that approximately 275 lb. of whole milk were replaced with 40 to 50 lb. of a dry milk substitute. Whole milk feeding was discontinued at 14 d. of age. Mixture VI, which has given the best results, was made up in per cent as follows: dried skim milk, 30; dried whey, 30; apple pomace, 10; linseed oilmeal, 10; dextrose, 9.738; oat flour, 5; dried brewers' yeast, 4.9; irradiated yeast, 0.1; stabilized vitamin A powder, 0.220; and trace minerals, 0.042. Average gains for ten calves on this mixture were 96.7 and 104.1% of the Ragsdale standard at 8 and 16 wk., respectively. A mixture containing dried banana meal promoted growth which closely paralleled the Ragsdale standard. A mixture containing 20% finely ground beet pulp gave poor results. Another mixture containing 20% of a high fat soya flour caused severe scouring and growth was subnormal. With a few exceptions calves on all mixtures have exhibited a rather rough appearance from the 3rd week through the 7th or 8th week, but after that age they grow at a rate approximately normal.

P13 Milk Replacements in the Rations of Dairy Calves.

J. B. WILLIAMS AND C. B. KNOTT, Pennsylvania State College.

Trials are being conducted to determine the value of various products such as dried skim-milk, dried whey, dried grain distillers solubles, dried blood flour, beet pulp and various other products in the development of milk replacements. The calves in these trials are limited to 50 lb. of whole milk including colostrum and receive the milk replacement in water at 100° F., beginning on the 5th day through the 56th day. Of the four formulas studied, the following has produced calves equal in appearance and rate of growth to calves receiving 300 lb. of whole milk: dried skim milk, 50; dried whey, 10; dried grain distillers solubles, 10; blood flour, 10; dried

brewer's yeast, 4.90; dextrose, 7.75; oat flour, 5.0; irradiated yeast (9F), 0.10; stabilized vitamin A (2,220,000 I. U./lb.), 2.20; and mineral mixture, 0.042. One replacement containing 20% beet pulp gave subnormal growth and resulted in a loss of hair, general muscular weakness, excessive lacrimation and papilledema. This condition was not corrected by the administration of Vitamin A, pantothenic acid, or biotin. Two other mixtures containing 10% beet pulp resulted in some loss of hair but gave normal growth.

P14 Diurnal Variations in Concentrations of Fat in Blood Plasma of Calves Fed Various Types of Oils. H. B. BARKER AND N. L. JACOBSON, Iowa State College.

Previous routine determinations of fat in the blood plasma of calves fed reconstituted milks containing various oils revealed that the highest values were from diets containing either crude or refined soybean oils followed, in order, by butter oil and hydrogenated soybean oil. Since the stage in the absorption cycle represented by blood samples collected 9 hr. after ingestion was unknown, possible differences in the rates of absorption of the respective oils were considered in the etiology of the responses.

The test diets used in ascertaining the diurnal changes of the fat levels in the blood plasma were reconstituted milks containing 3% of one of the following oils: crude soybean, hydrogenated soybean and butter. Each type of milk was fed at the rate of 10 lb./100 lb. body wt. daily to individual calves over a period of 3 consecutive weeks. In two trials, one after the 2nd week and the other after the 3rd, samples of blood were collected at 3-hr. intervals during the interim between feedings to determine changes in plasma fat values. The results from 12 such trials, involving six different calves on each oil revealed no marked differences in the form of curves; all increased to a maximum level during the first 6 hr. and subsequently receded to the initial level by the 12th hr. The differences in plasma fat concentrations resulting from ingestion of the different oils were similar throughout the period.

P15 The Hydrogen Ion Concentration and Dry Matter of the Feces of Young Dairy Calves Raised on a Limited Whole Milk—Dry Starter Method. R. E. JOHNSON, H. D. EATON, J. H. KRAMER, E. L. JUNGHER, W. N. PLASTRIDGE AND L. NEZVESKY, University of Connecticut and Storrs Agr. Expt. Station.

Diarrhea occurs frequently in young dairy calves and may be a predisposing factor in calf

mortality. The terms "diarrhea" and "scours" have been used subjectively in calf studies.

To evaluate these terms more objectively in future studies, pH and dry matter were determined on feces obtained from 15 calves which apparently were free from looseness of the bowels and which were raised under a limited whole milk-dry starter system. Feces samples were obtained from 1 through 14 d. of age, and in addition on the 18th, 21st and 28th days. At the first day of age pH values were between 5.4 and 6.2 and thereafter a gradual decrease in acidity occurred. Between the 14th and 21st days, a change from acidity to alkalinity occurred. At the 28th day, the pH values ranged from 7.2 to 8.4. In contrast to pH, a wide degree of variability occurred in the dry matter. In all calves, the dry matter increased to a maximum level at 3 to 4 d. of age. Thereafter, a marked drop occurred and the lower level was maintained throughout the remainder of the 28 d.

P16 The Influence of Pasture and Rumen Inoculation on the Establishment of Certain Microorganisms in the Rumens of Young Dairy Calves. W. D. POUNDEN AND J. W. HIBBS, Ohio Agricultural Experiment Station.

Rumen inoculations with cud materials from cows on pasture were given 6 of 12 calves which were fed milk and placed on lawn pasture at 4 d. of age. Rumen protozoa and certain bacteria, used as indicators of the presence of varieties characteristically associated with hay ingestion, readily were established in all inoculated calves. The bacteria were established in a relatively less degree in two calves which received grain supplement. Protozoa did not develop in the uninoculated calves. Some characteristic bacteria became established in four of the six uninoculated calves by 6 wk. of age, but were limited to one of the observed varieties and were relatively few in number. The incidence of diarrhea among these calves was low and did not appear to be influenced by the inoculations.

Characteristic rumen microorganisms became established only in relatively limited numbers in an uninoculated, 2-mo. old calf when it was turned out on pasture for 7 wk. with four inoculated calves of similar age. The marked difference in microorganisms was rectified following rumen inoculation. Prior to inoculation, this calf had persistent mild diarrhea while on pasture but gain in weight was similar to an inoculated twin.

P17 The Influence of Pasture and Early Rumen Development on the Changes in the Plasma Carotenoids, Vitamin A and Ascorbic Acid

and the Liver Storage of Carotenoids and Vitamin A of Young Dairy Calves. J. W. HIBBS AND W. D. POUNDEN, Ohio Agricultural Experiment Station.

As a measure of the influence of pasture and early rumen development in meeting the vitamin needs of calves, an experiment was conducted using 12 calves tethered during the day on a lawn pasture beginning at 4 d. of age. One-half of the calves were inoculated with cud material from older animals, which were eating pasture, and a 14% protein grain mixture was fed *ad libitum* to half of the calves. Three calves fed in the barn served as controls. Milk feeding was limited in all calves to the rate of 0.9 lb./10 lb. of body weight at birth. Blood samples were drawn for analysis on the 4th and 7th days of age and weekly thereafter for 6 wk. Ten calves were slaughtered at 42 d. of age and their livers were analyzed for carotenoids and vitamin A.

The calves on pasture maintained much higher blood levels and liver storage of carotenoids and vitamin A during the first 6 wk. after birth than the controls. Plasma carotenoids averaged 255 γ /100 ml. at 42 d. of age. The plasma ascorbic acid at 14 d. of age also was higher in the pasture fed groups.

Rumen inoculations were not shown to affect the blood or liver vitamin levels observed.

All calves grew well and no adverse effects due to the consumption of pasture were observed.

Data also are presented showing the changes in plasma carotenoids, vitamin A and ascorbic acid before and after turning five older calves (average 71 d. of age) out to pasture. These calves had been raised until the pasture period in the barn, three with and two without rumen inoculations.

It is concluded that good pasture grass, when available, can be utilized by calves, even at an early age, as an effective means of meeting their vitamin needs and as an economical source of other nutrients.

P18 Carotene Requirements for Young Dairy Calves. R. F. ELLIOTT¹, Cornell University.

Studies were carried out involving 19 Guernsey and 16 Holstein calves in an attempt to determine the amount of carotene necessary to promote normal growth, maintain adequate plasma levels and obtain liver storage of vitamin A. A daily intake of 3.2 mg. of carotene/100 lb. body weight was not sufficient for normal growth. This carotene intake did not maintain adequate plasma vitamin A levels or liver stores in Guernsey calves up to 60 d. of age, even though the calves were

¹ Present address: University of Kentucky.

allowed colostrum. The Holstein calves were able to make an average gain of 53 lb. in 60 d., even though the plasma vitamin A levels were below normal and no liver storage occurred. Daily intakes of 6 mg. of carotene for Holstein and 10 mg. for Guernsey calves for each 100 lb. body weight were sufficient to maintain only borderline plasma levels and slight liver storage of vitamin A. Normal growth occurred at these levels of intake.

P19 The Plasma Levels of Carotene and Vitamin A in Calves from Dams Milked Prepartum and in Calves from Dams Milked Postpartum. H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON, AND L. D. MATTERSON, University of Connecticut and Storrs Agricultural Experiment Station.

Prepartum milking results in a marked decrease in both the carotene and vitamin A content of colostrum. Since colostrum contributes a large proportion of the carotene and vitamin A ingested by the young calf, it is of value to know what effect prepartum milking of the dam has on these metabolites in the young calf.

A total of 36 calves, 18 of which were from dams milked twice daily for 10 d. prior to the due date and 18 of which were from dams milked only after calving, were used. All calves were fed weighed amounts of colostrum and milk from their respective dams for the first 7 d. after birth and herd milk thereafter. After the first week, all calves had free access to hay, starter and water. Blood samples were drawn at birth and at weekly intervals thereafter until 4 wk. of age.

There were no significant differences in the blood plasma levels of carotene and vitamin A at birth. However, for the remainder of the experimental period, 1, 2, 3 and 4 wk. of age, both the blood plasma carotene and vitamin A were significantly higher in those calves from dams milked postpartum ($P < 0.01$), as compared to those values for the calves from dams milked prepartum.

Prepartum milking results in significantly lower blood plasma levels of carotene and vitamin A in young calves.

P20 Effect of Type of Dispersion on Rate of Absorption of Carotene and Vitamin A by Dairy Calves. G. H. WISE, N. L. JACOBSON, R. S. ALLEN AND S. P. YANG, Iowa State College.

Carotene and vitamin A, respectively, were dispersed in a reconstituted milk, containing 10% non-fat milk solids and 3% hydrogenated soybean oil, and were fed to calves at the rate of 100,000

I.U./100 lb. body wt. Subsequent changes in the concentrations of carotene and vitamin A in plasma of blood collected at 3-hr. intervals over a period of 15 hr. were used as an index of absorption. Employing the nipple system of feeding calves, uptake of carotene from an oil concentrate (50,000 I.U./g.) in the milk was greater when dispersion was accomplished by homogenizing at 3,000 lb. pressure than when effected by stirring. A similar comparison of dispersions of a natural ester vitamin A oil (30,000 I.U./g.) revealed no difference between homogenization and stirring. The rate of absorption of vitamin A, however, was greater than that of carotene.

Vitamin A oil (100,000 I.U./g.) dispersed by means of an emulsifying agent (Tween 80)¹ was absorbed more readily and apparently to a greater degree than the unemulsified oil. The differences were more pronounced when given via nipple than when delivered by stomach tube into the rumino-reticular cavity. The slower absorption from the latter procedure substantiates previous observations indicating that the method of administering vitamin constituents affects the rate of absorption.

P21 Studies on the Site of Absorption and Conversion of Carotene to Vitamin A in the Dairy Calf. R. F. ELLIOTT², Cornell University.

An operative technic was used as a method for studying the sites of absorption and conversion of carotene to vitamin A in the dairy calf. Blood samples taken from various sites along the intestinal tract showed a seemingly significant increase in plasma vitamin A values following the ingestion of carotene concentrate. The carotene plasma values showed a marked decline in most cases in contrast to that expected if carotene was absorbed as such into the blood supply leading from the small intestine. The carotene values of fresh liver tissue from biopsy samples showed a corresponding increase but were smaller than the increase in vitamin A. Evidence was presented in support of the view that the intestinal wall is a site of conversion of carotene to vitamin A in dairy calves.

High-carotene blood plasma was given intravenously to two Guernsey and two Holstein calves to study further the function of the liver in carotene conversion. Since the intestinal tract was by-passed in this way, it was assumed that any increase in plasma vitamin A or in liver stores would be due to conversion of carotene to vita-

¹ Vitamin preparations supplied through the courtesy of Distillation Products, Inc., Rochester, N. Y.

² Present address: University of Kentucky.

min A in the liver. Decreases in the plasma carotene values occurred in Guernsey and Holstein calves with no rise in the plasma vitamin A values. The liver vitamin A values did not increase in Guernsey calves injected intravenously with high-carotene plasma. Large increases were observed in Holstein calves, but only equal to that observed in a control calf. No positive evidence was obtained to show that carotene in blood plasma is converted to vitamin A in either the Guernsey or Holstein calf.

P22 Calf Losses in a Dairy Herd Consisting of Five Breeds. E. E. ORMISTON, University of Illinois.

The losses of dairy calves even in well-managed herds limit the opportunity for selection of only the more desirable animals in a constructive breeding program.

The losses occurring in a herd of five breeds, from 1935 to 1947, inclusive, are reported. A total of 809 heifer calves was born, of which approximately 6% were abortions and stillbirths. Of the normal heifers born 24% died before they were 1 yr. of age. Seventy per cent of these calf losses occurred within the first 60 d. and in approximately one-third of the deaths, pneumonia was identified as one of the responsible agents. There was no significant difference in the losses between the five breeds.

The herd was maintained under what generally are considered good management practices and was free from contagious abortion. Accurate records were kept of the history of each calf with a clinical report of the cause of death in practically all instances.

P23 The Influence of Variations in Environmental Temperature and Thyroid Status on Growth and Feed Consumption in the Male Mouse. M. MAQSOOD AND E. P. REINEKE, Michigan State College.

Groups of young male mice were fed 0.1 or 0.2% thiouracil or several different levels of thyroprotein for periods of 3 to 4 wk. at environmental temperatures of 24 and 30° C. Their daily food and water consumption and weekly body weights were recorded throughout the experimental periods and compared with normal control values obtained under the same environmental conditions.

Thyroprotein, when fed as 0.025 and 0.05% of the feed at 24° C., caused an increase of 13 and 24% in body weight gain, respectively, and the mice consumed 20 and 30% more feed per day when compared with the control group. The increase in food and water consumption increased

with the increase in dosage of thyroprotein but there was no direct relation between the amount of feed consumed and the weight gained at 24 and 30° C. The mice fed 0.005% thyroprotein at 30° C. consumed 0.8 g. less feed/g. gain in body weight and gained 8 and 31% more weight when compared with the two control groups. Thiouracil administration caused a decrease in body weight gain and food and water consumption.

Raising the environmental temperature from 24 to 30° C. caused a tenfold reduction in the optimal thyroprotein dosage in the mouse, indicating that the demand for thyroxine is comparatively less at high than at low temperatures.

P24 Factors Affecting Heat Tolerance of Dairy Cattle.¹ R. E. McDOWELL AND R. A. HILDER, Bureau of Dairy Industry, USDA.

Studies have been conducted under controlled environmental temperature conditions with Holstein cows at Beltsville to determine the effect of stage of lactation and plane of nutrition on heat tolerance. In comparing dry cows and lactating cows at 65° F., there was no difference in body temperature between the two groups. At air temperatures of 90 and 100° F., there was little change in the body temperature of the dry cows, but there was a definite increase in body temperature in the lactating cow group. Greatest rises in body temperature are associated with higher feed intake at high environmental temperatures in both dry and lactating cows.

In these studies respiratory rate tended to parallel environmental temperature in all groups. Environmental temperature appeared to have no direct effect on pulse rate.

P25 The Comparative Heat Tolerance of Red Sindhi X Jersey and Other Breeds of Dairy Calves.¹ R. A. HILDER AND R. E. McDOWELL, Bureau of Dairy Industry, USDA.

The effect of high environmental temperatures on body temperature, respiratory rate and heart rate have been studied on four groups of calves: Red Sindhi X Jersey crossbreds, purebred Jersey, purebred Holstein and crossbreds of various combinations of European breeds. Part of the studies were carried out under natural climatic conditions at Beltsville during the summer of 1947, and part were done in a room in which air temperature was controlled. In their ability to maintain normal body temperature, the groups rank as follows:

¹ This work was done with funds from the Agricultural Research and Marketing Act of 1946.

lows: Red Sindhi X Jersey, purebred Jersey, crossbreds of European breeds, and purebred Holstein. The difference between the Red Sindhi X Jersey group and the purebred Jersey group was slight except during prolonged exposure to high environmental temperature. The Red Sindhi X Jersey group definitely was lower than the other groups in respiratory rate. In these studies environmental temperature appeared to have no direct effect on heart rate.

P26 Reactions of Dairy Cows to Higher Temperatures. S. BRONDY,¹ Missouri Agricultural Experiment Station.

The lowest temperature at which cows begin to show decline in milk production and feed consumption and rise in rectal temperature is between 70 and 85° F., varying with body size and milk-yield level—the greater the body size and milk yield the lower this “critical temperature.” The highest temperature used was 105° F., when chamber and skin temperature met, obviously an important biological constant; the rectal temperature then was between 106° F. (Jerseys) and 108° F. (Holsteins); milk production and feed consumption then virtually ceased.

Respiration rate increased in “acclimatized” cows from an initial 25/min. at 50° F. to maxima of 90 in Holsteins at 95° F. and 130 in Jerseys at 105° F.; in “unacclimatized” cows the maximal respiration rates of 118 in Holsteins and 155 in Jerseys were reached at 95° F.

Rectal temperature began to rise earlier (at 70° F.) and the rate of rise was steeper in the larger cows; its rise was least in cows with the greatest rise in respiration rate; its rise was least in cows that drank the most water. One cow increased her water consumption (with corresponding increase in urine output) from 10 gal. at 50° F. to over 45 gal. at 100° F.

A sharp rise (100%) in blood creatinine was found. A decline in blood cholesterol resulted. A decline (40%) in heat production occurred with increasing temperature above 80° F.

P27 The Effect of Increasing Environmental Temperatures on the Composition of Milk.

J. W. COBBLE AND A. C. RAGSDALE, University of Missouri.

Changes in the composition of milk where cows are subjected to high environmental temperatures were studied on a group of ten cows in the Psychroenergetic Laboratory at the Univer-

sity of Missouri. Five cows were used as controls and five were subjected to various temperatures over a 5-mo. period. The temperature on the experimental cows, three Jerseys and two Holsteins, was increased systematically from 50 to 105° F. by 5 to 10° F. During the last 14 d. of the experiment the control cows were subjected to a rapid increase in temperature up to 100° F. Some 850 samples of milk were collected and determinations made for total solids, fat percentage, solids-not-fat, lactose, chloride and nitrogen.

Data from these studies indicate a rise in the fat percentage, total solids and chlorides with an increase in temperature above 80 to 90° F., varying with breed and individual. Solids-not-fat, lactose and nitrogen, to a lesser extent in the Jerseys, showed a downward trend at the same temperature levels. All constituents studied returned to approximately normal levels with a return to 60° F. reading.

In the rapid change of temperatures up to 100° F. over a 14-d. period, extreme increases were observed in the total solids, solids-not-fat, fat percentage, chlorides and nitrogen. Lactose dropped to a low level.

Results obtained in composition of milk indicate breed differences, as well as an individual difference in ability to withstand high environmental temperatures.

P28 The Influence of Temperature on the Carotenoid and Vitamin A Content of Milk Fat.

O. T. STALLCUP AND A. C. RAGSDALE, University of Missouri.

Studies have been made on the effects of environmental temperature on the carotenoid and Vitamin A content of milk fat of four Holstein and six Jersey cows in the Psychroenergetic Laboratory at the University of Missouri. Two Holsteins and three Jerseys were kept in a chamber in which the temperature was raised from 50 to 105° F. over a 5-mo. period. Two Holsteins and three Jerseys serving as controls were kept at a temperature approximating 50° F. for the duration of the experiment. The animals were fed the regular grain mix used in the University herd. Cod liver oil furnishing 75,000 units of Vitamin A and 10,000 units of Vitamin D was added at the time the animals were changed to 80° F. The animals were fed 2 lb. of beet pulp/day and alfalfa hay *ad libitum* as the sources of roughage. The carotenoids and Vitamin A were determined by the method of Boyer *et al.* Milk samples were taken at approximately weekly intervals throughout the trial.

There was variation in both the experimental and the control groups with regard to the caro-

¹A summary of results prepared for publication (Missouri Research Bull. 423, 425, 433, 435, 436) by staff members of the Climatic Project on which the Missouri Agricultural Experiment Station and the USDA (BPISAE) are cooperating.

tenoid content of the fat. In most cases these variations tended to follow the consumption of hay rather than the pattern of ambient temperatures. The Vitamin A content of the milk fat was less variable, there being little difference in the values of either group except in the case of some of the experimental animals when they were taken from the hot chamber and feed consumption was increased markedly.

P29 The Carotene Requirements of Guernsey Cattle for Reproduction (Progress Report).
A. H. KUHLMAN AND W. D. GALLUP, Oklahoma A. and M. College.

If the carotene intake per pound body weight during the last 90 d. before calving is used as a basis for determining the carotene requirements of Guernseys for reproduction, the 32 calving records now available show extremely large and seemingly inconsistent variations between individual cows. In 20 cases in which the average daily carotene intake during the last 90 d. before calving ranged from 38 to 81 γ per lb. body weight, reproduction was unsatisfactory in more than one-half of the cases at all levels within the range of carotene intake in this group. Of the twenty calves, only nine were normal at birth, four had a constricted optic foramen, three were born prematurely, one died of convulsions, and three were extremely weak. Twelve cows with carotene intakes ranging from 83 to 158 γ daily during the last 90 d. before parturition produced eight normal calves, two weak calves which died and two dead calves.

No positive conclusions can be drawn from information available now except that, in general, the carotene requirements for Guernseys for reproduction appear to be much higher than for Jerseys and there seem to be much greater individual variations in the Guernsey breed than were found in Jerseys.

P30 Vitamin A Absorption Studies in Ruminants. R. P. NIEDERMEIER, V. R. SMITH AND L. H. SCHULTZ, University of Wisconsin.

Changes in plasma vitamin A levels were used to study absorption of this vitamin from the abomasum, small intestine and large intestine of goats. Massive doses of aqueous dispersed vitamin A were administered by direct injection into the part of the tract being studied, and when necessary that portion of the tract isolated by ligation and severance. Controls were treated the same way except for injection of vitamin A. Vitamin A was determined on pre-treatment blood samples and on samples taken at 2-hr. intervals for the first 10 hr. of the post-treatment period.

After the administration of 250,000 I.U. of vitamin A, the following blood plasma increases in vitamin A levels were obtained: An increase of 10 γ per cent when injected into the abomasum, an increase of 20 and 50 γ per cent in two trials on the small intestine, and 14 γ per cent in the large intestine. In all cases the peak was reached within 10 hr. after treatment, with a drop to near pre-treatment levels in 24 hr. With oral administration the maximum increase of 9 γ per cent was reached in 12 hr. with no appreciable drop in 72 hr.

P31 Relation Between the Carotene in the Feed and the Vitamin A Potency of Butter. H. G. WISEMAN AND J. B. SHEPHERD, Bureau of Dairy Industry, USDA.

The vitamin A potency of butter in I.U./lb. has been shown by results at Beltsville to be, in general, related to the carotene intake by the equation $Y = 12,912 + 8,937x$, $x = \text{Log intake (mg./day)}$. From this equation it is evident that even for "winter butter" a much greater carotene intake (480 mg./day) is required than for any other normal function of the cow. To improve the vitamin A potency of "winter butter" for human use much better methods of carotene preservation in forages are needed, yet methods which for the farmer are economically feasible.

The losses in carotene in the harvesting of comparable lots of alfalfa as field-cured hay, barn-dried hay and as silage have been measured during the past 3 yr. at Beltsville. Silages have shown nearly 9 times as much carotene as field-cured hay not exposed to rain, and 20 times more carotene than that which was exposed. Silage showed less loss in dry matter and protein than field-cured or barn-dried hays. Butter obtained from cows fed this silage had three times the vitamin A potency of butter from cows fed U. S. no. 2 field-cured hay.

If high quality alfalfa silage can be produced regularly it appears economically practical to raise the vitamin A potency and yield of milk or butter during winter feeding.

P32 Further Studies on the Relation of Soybeans to the Vitamin A Requirements of Dairy Cattle. M. F. ELLMORE AND J. C. SHAW, University of Maryland.

Calves on a ration containing 30% raw soybeans and receiving 48 γ of carotene/lb. of body weight exhibited a 40% decrease in plasma carotene and 30% decrease in plasma vitamin A. Calves on the same ration but receiving 64 γ of carotene/lb. of body weight exhibited a similar decrease in plasma carotene and vitamin A. The

addition to soybean oil of a combination of N.D.-G.A., Tenox 2, and quercetin sulfonic acid at a level of 0.4% of the oil did not prevent the adverse effect of soybeans.

P33 Role and Sources of B₁₂ in the Normal Mammal. A. M. HARTMAN, L. P. DRYDEN AND C. A. CARY, Bureau of Dairy Industry, USDA.

The authors reported some years ago, as the result of work with rats, that a chemically still-identified nutrient (*X*) occurs in milk, non-fat milk solids, cheese, commercial casein, liver extracts and leafy foods and feeds (roughages), but is practically absent from yeast and grains; that this factor is required for growth, development, reproduction and lactation; that growth without it is particularly poor on diets containing lactose; and that high levels of protein are very deleterious, may even be lethal, when fed in diets deficient in *X*.

Insofar as growth is concerned, B₁₂ now has been shown to function like *X* even with animals on high protein diets; the relation of B₁₂ to the utilization of protein, especially in relation to the utilization of high-protein diets, suggests a fundamental function of this vitamin in the normal mammal.

As a result of this work it is obvious that the foods and feeds with *X* activity possess B₁₂ potency, but a B₁₂ deficiency in our animals may be overcome by feeding crystalline B₁₂, or materials like liver extracts which contain B₁₂ in a dialyzable form, or milk or the above milk products or leafy foods or feeds which contain a chemically still-unidentified, non-dialyzable material which produces the same result in the animal as B₁₂ or diets (like one containing an excessively high level of riboflavin) which induce the development of bacteria in the animal that synthesizes a B₁₂-active material.

Evidence indicates that cows ordinarily may synthesize enough B₁₂ to maintain the B₁₂ potency of the milk. There are suggestions, however, that some results on protein requirements of milking cows may have to be reconsidered in this connection.

P34 The Vitamin D Content of Roughages. G. C. WALLIS, C. A. SMITH AND R. H. FISHMAN, Standard Brands and Agricultural Experiment Stations of Florida, Illinois, Kansas, Michigan, New York, North Carolina, South Dakota, Texas and Washington and The California State Polytechnic College.

A nation-wide survey of the vitamin D content of roughages has been made. Sixty-two samples of hay and other roughages representative of the

kinds produced and curing methods used in the various sections of the country are included. Hay samples were graded and classified by the USDA.

The results revealed striking and unpredictable variations in the vitamin D content of roughages. The lowest value obtained in this study was 59 U.S.P. units of vitamin D/lb. for a sample of sun-cured oat and vetch hay. The highest was 1,407 units for a sample of sun-cured peanut hay. Sun-cured alfalfa hay varied from 64 to 1,044 units of vitamin D per lb., a 16-fold variation. Dehydrated alfalfa meals ranged from 82 to 268 units/lb. Strangely enough, the low value for sun-cured alfalfa hay was less than the low value for dehydrated alfalfa meal. Sun-cured prairie hay varied from 499 to 681 units/lb. mow-cured soybean hay from 163 to 681 and silages of various kinds from 61 to 105 units/lb. of wet silage. Information also will be presented on the vitamin D content of sun-cured timothy hay, clover, soybean and lespedeza hay, various kinds and grades of mixed hays, sorghum fodder and winter range grasses.

These results emphasize the fact that wide and unpredictable variations exist in the vitamin D content of roughages. Many factors are concerned in determining their vitamin D content besides the extent of sunshine exposure during curing. Much careful research is needed yet to discover these factors and to properly evaluate their effect on the vitamin D content of roughages.

P35 The Effect of the Proportion of Roughage in the Ration on the Growth of Dairy Heifers. K. E. HARSHBARGER AND G. W. SALISBURY, University of Illinois.

Differences in growth of 15 Holstein heifers fed rations containing from 55 to 94% roughage have been measured. Five rations with three heifers on each have been tested. The four experimental rations were made up principally of timothy hay, soybean meal and corn. The control ration was made up of clover hay, corn silage and limited grain. The experimental rations contained from 48.2 to 61.5% TDN and approximately 7.2% digestible protein. The timothy hay and grain were ground and mixed together in order that all components of the rations would be consumed in the same ratio.

The average daily gain in live weight at the end of 12 wk. was above normal for all rations except the experimental ration containing 94% timothy hay. However, the TDN consumed per pound of gain in live weight above the estimated maintenance requirement was approximately the same for all rations except the experimental ration containing 55% timothy hay. The ration containing 70% timothy hay produced the largest

rate of gain and required the least amount of TDN/lb. of gain.

P36 Preliminary Report on the Influence of Soil Fertility on the Health, Reproduction and Milk Production of Dairy Cows. C. W. DUNCAN, K. M. DUNN, R. E. ELY, S. T. DEXTER AND C. E. MILLAR, Michigan Agricultural Experiment Station.

A long-range experiment was started in 1945 to study the characteristics of various species of plants when grown on natural soil highly depleted of mineral nutrients, and on the same soil with large additions of lime and mineral fertilizers. The products from a 200-acre farm so handled were fed to two groups of dairy cows. Since the two rations must be composed of the same species, and since forage legumes such as the clovers and alfalfa would not grow on the depleted soil, grass hays were grown, together with corn, wheat, oats and soybeans as concentrates. At the outset, it was recognized that this would not constitute a thoroughly satisfactory dairy ration, due to the lack of a legume hay, and that, in agricultural practice, alfalfa or clover would be grown on the improved soil.

The chemical composition of the crops grown on the fertilized and depleted soils has been determined for each crop year. The oats, corn, and soybeans show a remarkable uniformity in composition from year to year. In the first harvest year, the hay from the fertilized fields was stemmy and was not consumed readily. To correct this difficulty, the amount of nitrogen fertilizer was decreased and the hay was cut earlier than on the depleted fields. The total digestible nutrient and digestible protein intakes are now approximately equal for both groups. The milk and fat production records of the cows receiving the unfertilized hay definitely were higher in the first lactation period than those of the cows receiving the fertilized hay, but this difference has diminished. No difference can be noted in the general well-being of the cows on the two rations. Herd health has not been a problem with these cows. At the present time the data show a slightly higher number of services per conception for the cows receiving the feed grown on the fertilized soil, although little significance can be attached to these at this time.

Systematic chemical analyses have been made on the colostrum, milk and blood of all the cows since the beginning of the experiment. These data show no differences that would indicate a nutritional superiority of the crop species grown in highly fertilized soil over the same crop species grown on the depleted soil. As would be antici-

pated, crop yields on the fertilized soil were far greater than those on the depleted soil.

P37 Conservation of Nutrients and Feeding Value of Wilted Silage, Barn-cured Hay and Dehydrated Hay. R. E. ELY, L. G. SCHÖENLEBER, J. B. SHEPHERD, H. G. WISEMAN, C. G. MELIN, W. H. HOSTERMAN AND R. E. WAGNER, Bureau of Dairy Industry, BPISAE, PMA, and USDA.

Comparable plots of second cutting 1948 forage were harvested and stored as wilted silage, barn-cured hay and dehydrated hay. The forage was principally alfalfa with a small percentage of grass. All forage was harvested with a field chopper. Supplemental heat was used in barn hay drying. One-half of each kind of forage was cut and harvested on July 13, 14 and 15, and the balance on July 19, 20 and 21. Part of the forage harvested by each method was rained on before being stored. The forages all were of about equal quality with an official grade of U. S. no. 2 leafy alfalfa light grass mixed hay.

Nutrient preservation in the wilted silage, barn-dried hay and dehydrated hay was, respectively: dry matter 77, 79 and 84%; protein, 77, 77 and 81%; and carotene, 17, 15 and 25%. Average daily milk production per cow on wilted silage, barn-dried hay and dehydrated hay was 33.9, 36.1 and 35.2 lb., respectively. Initial production was about the same on wilted silage and dehydrated hay, and about 1.5 lb. higher on the barn-dried hay. The 30-d. declines in production were 10.2, 7.8 and 7.1%, respectively. The labor and equipment requirements were about equal for the wilted silage and barn dried hay, but the labor required for the dehydrated hay was somewhat higher.

P38 Observations on Time Required for Dairy Cows to Eat Grain, Silage and Hay. K. E. HARSHBARGER, University of Illinois.

The principal objective in this study was to determine the average rate at which dairy cows eat grain, silage and hay. Time studies were completed on Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey cows.

The average rates of eating ranged from about 2 to 3 min./lb. for grain, 1.75 to 2.75 min./lb. for silage and 7 to 16 min./lb. for hay. For every type of feed, the rate of eating was highest for Holsteins and lowest for Jerseys, with other breeds intermediate. In addition to breed differences, there are many other factors that affect the rate of eating feeds by dairy cows.

Information on the time required by cows to consume grain is of considerable practical im-

portance in connection with the design and operation of milking parlors. When cows are milked in a milking parlor by the rapid milking procedure, they generally do not have sufficient time to eat grain allowances during the actual milking process.

P39 How Hay Feeding to Cows on Pasture Affected Milk Production and Body Weight. D. M. SEATH, University of Kentucky.

No appreciable increase in milk production or body weight change resulted from feeding alfalfa hay free-choice to milking Holstein and Jersey cows on pasture during a 45-d. experimental period from July 17 to August 30, 1948. Two experimental groups of 5 cows each received hay and were compared to a third five-cow control group not receiving hay while grazing on orchard grass-alfalfa pasture. Not over 20% of the herbage consisted of alfalfa and the grazing took place following the harvesting of the early spring growth for silage and hay. Hay consumption varied from slightly less than 6 lb. per head daily during the first 13 d. of the experiment, when receiving first cutting alfalfa hay, to slightly more than 14 lb. during the last 32 d. of the experiment when second cutting alfalfa was being fed. Relatively dry weather during the last 2 wk. of the experiment caused the pasture herbage to appear dry and lacking in succulent growth. In spite of this, the group not receiving hay produced as well as did the groups receiving hay during this period. All three groups produced at a lower level than did ten comparable cows within the regular experiment station herd grazing bluegrass pasture. Besides the difference in pasture, this latter group had access to more ample shade than was available to the three five-cow groups.

P40 A Method for Estimating the Feed-replacement Value of Pasture Forage. W. B. NEVENS, R. W. TOUCHBERRY AND J. A. PRESCOTT, JR., University of Illinois.

This investigation had for its objective the design of a simple method for the measurement of the feed-replacement value of pasture herbage. Fifteen pairs of high-producing cows were selected. The cows of a pair were nearly alike in weight, stages of lactation and gestation and level of milk yield. One cow of each pair was assigned at random to the "pasture group" and the other to the "dry-lot group". The pasture group was allowed access to a pasture of known area and was given grain mixture as the only supplementary feed. The dry-lot group received only silage, alfalfa hay, beet pulp and the same kind of grain mixture as that given the pasture group. The experiment lasted 18 wk.

The production of the two groups was not significantly different. The method of calculating the feed-replacement value of the pasture is to find the difference between the amounts of feed, other than pasture, given the two groups of cows. For this experiment, the difference amounted to 3,554 lb. silage, 1,935 lb. hay, 169 lb. beet pulp and 510 lb. grain mixture. Dividing each of these amounts by 38.38, the yield per acre of FCM (fat-corrected milk) in cwt., gives the feed-replacement value/100 lb. FCM.

P41 Distribution of Intravenously Injected Radioactive Phosphorous (P^{32}) in the Body of the Dairy Cow. N. P. RALSTON, MAX KLEIBER, A. H. SMITH AND A. L. BLACK, University of California.

A venous catheterization technic has been adapted for accurate injection of materials into the blood stream of dairy cows. This method allows the drawing of frequent blood samples over prolonged periods. A series of experiments has been conducted, concerning phosphorous metabolism, employing this method.

Two of these experiments were terminated by slaughtering the cows in order to study the distribution of the injected radioactive phosphorous in the tissues.

P42 The Effects of Lactose Feeding on Lactase Production.¹ JESSIE FISCHER, T. S. SUTTON, J. L. LAWRENCE, H. H. WEISER AND G. L. STAHLY, Ohio State University.

Three groups of rats were fed rations containing carbohydrate as follows: (I) 67% cornstarch; (II) 31.6% lactose (supplied by non-fat dry milk solids) plus 32.8% cornstarch; and (III) 31.6% USP lactose plus 35.4% cornstarch. Rats in groups II and III exhibited diarrhea. Diarrhea disappeared entirely in group III but occasionally was observed in group II during a 6-mo. feeding period. The rats then were killed and the total lactase activity of the small intestinal mucosa of each rat was determined. In 4 hr. the following average percentages of lactase were hydrolyzed: (I) 37.6%, (II) 48.2%, and (III) 42.7%. These differences were not significant.

In another experiment, three groups of rats were fed rations containing 67% carbohydrate as follows: (I) 67% cornstarch, (II) 15% USP lactose plus 52% cornstarch, and (III) 35% USP lactose plus 32% cornstarch. Rats from each group were killed and total small intestinal lactase determined at 0, 1, 4, 8 and 18 wk. No relationship between lactase concentration and lactose feeding at these two levels was found.

¹ Research supported by the American Dry Milk Institute, Inc.

These results considered in conjunction with a study of lactase activity of the cecal contents of some of these same rats reported elsewhere¹ indicate that any significantly greater lactase activity in response to lactose feeding is microbiological rather than mucosal in origin. However, the available data are not conclusive proof that the disappearance of diarrhea with continued lactose feeding is a result of microbiological changes alone.

P43 Blood Sugar Studies in Relation to Ketosis in Ruminants. L. H. SCHULTZ, V. R. SMITH AND H. A. LARDY, University of Wisconsin.

This study was undertaken in an effort to obtain additional information regarding the relationship between blood sugar and blood ketone levels in ruminants. Goats were used in all of the studies. By means of daily injections of 80 units of protamine zinc insulin it was possible to maintain blood sugar levels below 30 mg. % for a week with no increase in blood ketones or particularly adverse effects upon the animal. Intravenous injection of alloxan at the rate of 90 mg./kg. of body weight caused an initial hyperglycemia, then a hypoglycemia and finally a marked and permanent hyperglycemia. There was a gradual but marked increase in blood ketones. Daily subcutaneous injections of 1 g. of phlorhizin for a period of 1 wk. resulted in the excretion of approximately 40 g. of glucose daily but caused no marked changes in either blood sugar or blood ketone levels. Oral administration of 15 g. of ketogenic fatty acids (particularly butyric and caproic) caused marked but very transitory changes in blood sugar levels. First there was a hyperglycemia, then a hypoglycemia and finally a somewhat longer period of hyperglycemia. The lowered blood sugar immediately followed the peak of ketone body production.

P44 Biochemical and Histopathological Studies of Fasting Ketosis and Spontaneous Ketosis of Cows. J. C. SHAW, P. V. SAARINEN, B. C. HADJIJOLOS AND E. C. LEFFEL, University of Maryland.

Most of the biochemical alterations observed in the blood and various organs of cows with spontaneous ketosis can be produced by fasting cows in the early postpartal period. All the blood lipid fractions are low in both fasting and spontaneous ketosis. The plasma organic acid-soluble phosphorus is low in both cases. The inorganic phosphorus varies widely in both cases. Phosphatase values decrease in both fasting and

spontaneous ketosis. Ca, Na, K and Cl values are normal in both cases. In the early stages of spontaneous ketosis the liver fat often approaches normal values. The fatty liver observed in the later stages of spontaneous ketosis appears to be due primarily to fasting. The increase in the liver fat in both fasting ketosis and the later stages of spontaneous ketosis appears to be due mainly to glycerides. The ester cholesterol increases in both cases but tends to be higher in spontaneous ketosis. The total cholesterol in the liver varies within normal limits in both cases. Of particular significance is the fact that at the same low level of blood glucose, ketonemia is much more marked in spontaneous ketosis than in fasting ketosis. The liver glycogen is depleted markedly in both cases. Cows exhibiting fasting ketosis do not show clinical symptoms known to be associated with spontaneous ketosis though the blood glucose values may be very low. In fasting ketosis as well as in spontaneous ketosis, the liver, kidneys, and all of the endocrine glands exhibit fatty phaneriosis. In the later stages of spontaneous ketosis there is a marked degeneration of the epithelial cells of the various organs and endocrine glands, and severe damage to the convoluted tubules of the kidneys always has been observed. In most cases of spontaneous ketosis significant alterations have been observed in the anterior lobe of the pituitary. Also, in spontaneous ketosis the adrenal glands always are enlarged, with the degeneration of the epithelial cells being most marked in the zona glomerulosa.

P45 Standards for Growth in Weight of Jersey Heifers. C. A. MATTHEWS AND M. H. FOHRMAN, Bureau of Dairy Industry, USDA.

The average weights of 378 Jersey heifers, excluding twins and inbreds, born and raised in the Beltsville herd were used in preparing a standard of normal growth by 10-d. periods from birth to 365 d. of age. Average weights on 360 of these heifers were used in preparing a standard by months from birth to 21 mo.

Average weights at birth, 90, 180 and 365 d. were 55.6, 137.9, 277.0 and 519.3 lb., respectively, for the standard by 10-d. periods, and 55.7, 138.3, 277.1 and 519.7 lb., respectively, for the standard by months. The average weight at 18 mo. was 660.1 lb.

Slight irregularities in the growth curve for average weights were smoothed out by the fitting of 4th degree orthogonal polynomials to produce the standards for weights at specified ages and gains in weight during specified periods. The changes in the standard deviations with increase in age were smoothed out in the same manner.

¹ Society of American Bacteriologists, Proceedings of Annual Meeting, 1949.

Factors which divide a normal distribution into ten sections with an equal number of items in each were applied to these estimated standard deviations to provide a system for grading individual animals on the extent of the differences between their weights and the standard.

P46 The Value of Wood Molasses for Growth of Dairy Heifers. T. H. BLOSSER, G. W. SCOTT, R. E. ERB AND A. O. SHAW, State College of Washington.

Ten heifer calves (eight Holsteins and two Jerseys) ranging in age from 3 to 10 mo. were divided into two groups of five each. Both groups received chopped alfalfa hay and enough grain so that they were being fed total digestible nutrients at a level 5% below Morrison's (20th ed.) minimum standards for growing dairy cattle. In addition, one group was fed 2 lb. of wood molasses daily for the first 8 wk. (1st phase) of the experiment and 4 lb. of wood molasses daily for the next 7 wk. (2nd phase).

The wood molasses-fed group gained 1.22 lb./d. during the 1st phase and 1.37 lb./d. during the 2nd phase of the experiment. The control group gained 0.94 lb. daily during the 1st phase and 1.06 lb. daily during the 2nd phase. These differences were highly significant. The total of 1,540 lb. of wood molasses fed during both phases produced 155 lb. more gain in the wood molasses-fed than in the control group. Thus, 10 lb. of wood molasses produced 1 lb. of gain. There was no significant difference between the two groups in increase in height at the withers.

The major problem encountered was in regard to the palatability of the wood molasses. The calves did not relish it, although as the trial progressed they seemed to become more accustomed to its taste.

It is concluded that wood molasses is of value for growth of dairy calves.

P47 Effect of Various Milking Procedures, Prepartum and Postpartum, on Composition of Mammary Secretions. D. B. PARRISH, F. C. FOUNTAINE, G. H. WISE, F. W. ATKESON AND J. S. HUGHES, Kansas Agricultural Experiment Station.

Observations were made on composition of mammary secretions obtained by various milking procedures. Procedures used were: normal postpartal milking; partial postpartal milking of one-half of the udder; milking of cows and heifers commencing 8 to 12 days prepartal; milking one-half the udder throughout the gestation period, followed by normal milking postpartum; and single milkings 3 to 12 d. after commencement

of the normal dry period. Except in the latter case, milkings were made twice daily. Oxytocin was used in some of the trials.

Changes in the secretions were followed by determinations of quantities produced, specific gravity, and concentrations of fat, total solids, ash, lactose, protein, vitamin A, carotenoids and tocopherols. Observations also were made of condition of the udder and effect on later production of milk.

Cows and heifers that were milked commencing 8 to 12 d. prepartal produced secretions in the early stages that resembled colostrum and rapidly assumed the characteristics of normal milk in subsequent milking; only small, if any, further changes were noted at time of calving. Similar results were noted in the case of cows milked throughout the whole gestation period when quantities of secretions obtained totaled several pounds daily, whereas cows that tended to go dry in spite of continuous milking produced secretions at time of calving that had many of the characteristics of colostrum. Cows milked a single time 3 to 12 d. after commencement of the normal dry period frequently produced secretions in the early stages having a specific gravity lower than normal milk. No marked differences were obtained in the nature of the secretions when oxytocin was used.

Effect of the experimental procedures on content of various constituents of the mammary secretions and on condition of the cow will be presented and relation of the findings to theories of colostrum formation will be discussed.

P48 Some Effects of Prepartum Milking on the Performance of Cows and Calves. R. A. ACKERMAN, G. HYATT, JR. AND A. H. VAN LANDINGHAM, West Virginia University.

Forty-five Ayrshire cows and first-calf heifers were divided at random into a prepartum and control group. Of the 24 animals milked prepartum an average of 9 d., six produced less than 2 lb. the day preceding parturition; four 2 to 10 lb.; five 11 to 20 lb.; and nine over 20 lb.

Based on the total nitrogen and vitamin content, normal milk was produced at parturition by cows yielding over 20 lb. on the day before calving.

On the average, all prepartum groups reached their peak of production slightly later and maintained their production at near peak levels over a longer time than did the control group.

Daily observations of the amount of udder congestion and lymphatic edema, both before and after parturition, were recorded. There was considerable variation within groups. The results

indicated, however, that prepartum milking neither reduced the amount of congestion or edema nor the length of time required for the udder to become normal.

Calf feeding and management differed only in that calves from prepartum groups were given more cod liver oil the first 3 d. than those from dams in the control group. All calves received their dams' milk the first 4 d. No calf mortality or serious scours occurred. The average rate of gain of calves in all groups was satisfactory.

P49 The Effect of Prepartum Milking on the Ascorbic Acid and Riboflavin Content of Colostrum at Parturition. A. H. VANLANDINGHAM, C. A. FLANDERS AND R. A. ACKERMAN, West Virginia University.

Six cows, four Ayrshires and two Holsteins were milked 8 to 18 d., average 14 d., before parturition. Production on day before parturition varied from 10.3 to 24.6 with an average of 21.4 lb.

Wide variations were observed in the total nitrogen, ascorbic acid and riboflavin content of colostrum produced by cows milked before parturition. The composition of the colostrum at parturition was related to the level of production and the total amount produced before parturition.

Total nitrogen in colostrum on day of parturition averaged 727 mg./100 ml. Milk produced by 11 cows not milked prepartum averaged 737 mg. total nitrogen on the 3rd day following parturition.

Ascorbic acid decreased from an average of 18.5 mg./l. 3 d. before parturition to 4.7 mg. on the day of parturition and to 4.3 mg. on the 3rd day following parturition.

Riboflavin decreased from an average of 6.53 mg./l. 3 d. before parturition to 2.70 mg. on the day of parturition and to 1.90 mg. the 3rd day following parturition.

P50 Effectiveness of Penicillin Infusions in Eliminating Mastitis Infections in the Bureau of Dairy Industry Herd. W. W. SWETT, L. A. BURKEY, CECELIA R. BUCKNER AND P. C. UNDERWOOD, Bureau of Dairy Industry, USDA.

Beginning in 1944, penicillin was administered to a limited number of udder infections in the Bureau's dairy herd at Beltsville. At the outset the total dosage was 50,000 units. In some cases it was administered as a single infusion. In other cases 25,000 units was administered once daily on 2 consecutive days. About 65% of the streptococcal and one third of the staphylococcal infections were eliminated by these treatments.

From April, 1946, through December, 1947, penicillin was used exclusively. During this period each treatment consisted of two infusions daily on 2 consecutive days and the total number of units for the treatment was increased to at least 100,000. More than 90% of all streptococcal infections and 85% of all staphylococcal infections were eliminated when this four-infusion treatment procedure with increased dosage was followed.

The nature of infection found in this herd differed materially from that usually reported by other investigators. Only about 10% of the infections were caused by *Streptococcus agalactiae* and only 55% by streptococci of all kinds. Staphylococci accounted for more than 30% and coliform bacteria, pseudomonads, yeasts and cocci for the balance.

P51 The Incidence and Relative Severity of Infections of Different Organisms in Mastitis.

L. A. BURKEY AND CECELIA R. BUCKNER, Bureau of Dairy Industry, USDA.

The view still widely accepted that *Streptococcus agalactiae* is the principal infecting organism in mastitis is not borne out from the standpoint of either prevalence or severity of mastitis in results obtained in the herds maintained by the Bureau of Dairy Industry at Beltsville. Studies during the last 4 yr. have shown that *S. agalactiae* was associated in only 7 to 15% of the infections of mastitis, whereas *Streptococcus uberis* was found in 19 to 30% and hemolytic staphylococci in 21 to 38% of the infected quarters. Results indicate that incidence of infections of different organisms is related in part to the ease of their elimination by treatment.

Of the seven species of bacteria encountered in mastitis, at least 20 distinct strains were found associated with these infections. Studies on the severity of mastitis, judged by the leucocyte count and the percentage of chlorides of the fore-milk, indicate that most of these strains are capable of causing severe infections of mastitis. However, these studies indicate that certain strains of *Streptococcus dysgalactiae* and *S. uberis*, enterococci and streptococci of the viridans group usually were associated with relatively mild infections. Likewise they indicate that mixed infections of strains of *S. agalactiae* and certain strains of *S. dysgalactiae* and *S. uberis* with hemolytic staphylococci were found associated with less severe infections. However, such a measure of the degree of severity is not an adequate basis for predicting the effectiveness of treatment.

P52 A Study of the Reliability of Various Diagnostic Tests and the Efficiency of Certain

Therapeutic Measures in the Control of Mastitis. C. P. MERILAN, H. A. HERMAN, J. E. EDMONSON, K. L. TALLMAN AND O. S. CRISLER, University of Missouri.

A study of the reliability of various diagnostic tests and the efficiency of certain therapeutic measures in the control of mastitis has been made on the University of Missouri dairy herd over a 10-yr. period. A combination of the Hotis test and microscopic examination of incubated milk samples was used as a standard. The leucocyte count detected 71.92% of the positive samples and 84% which were negative to the Hotis and microscopic tests. The chloride test detected 53.5% of the positive samples and 78.4% of the negative samples. Agreement on 33.08% of the positive samples and 9.11% of the negative samples was shown in 1,852 comparisons of the bromothymol blue test with the Hotis and microscopic tests.

The fact that many cows affected with mastitis undergo "natural recovery" is recognized. In this study, approximately 38% of the untreated cows recovered.

The effectiveness of various treatments for mastitis was studied in 289 infected quarters of 140 cows. Only animals with at least two tests after treatment are included in this study. Treatment of 82 quarters with sulfanilamide in iodized mineral oil resulted in 73.17% of the quarters becoming negative to the Hotis and microscopic tests and 69.35% of the 186 infected quarters given penicillin infusions were negative within an average of 29 d. after treatment. Treatments with 4:4 diamino-diphenyl sulfone and Tyrothricin also were studied and results will be reported in detail.

P53 Preliminary Observations on the Biochemical and Serological Characteristics of Coliform Organisms Isolated from Cases of Acute Mastitis. J. C. OLSON, JR., I. A. SCHIPPER AND M. E. SCHMITZ, University of Minnesota.

The object of this study was to obtain information regarding the heterogeneity or homogeneity of coliform bacteria isolated from cases of acute bovine mastitis. Consequently, extreme care was taken to exclude external coliform contaminants. In addition, all isolations were made from poured plates on which a large number of coliform colonies occurred. Sufficient colonies were picked from individual plates to make reasonably certain that representative cultures of types present on the plates were obtained. No evidence was obtained, during the study of any of the cases, which would indicate that organisms other than coliform were etiological agents. All cultures were classi-

fied according to *Bergey's Manual of Determinative Bacteriology* and according to Parr (Bact. Rev., 3: 1-48. 1939). Seventy-six cultures were isolated and classified. The cultures could be segregated into two sharply defined biochemical groups. The organisms of one group, consisting of 64 cultures isolated from various quarters of six cows, were typical of *Aerobacter aerogenes*. The majority of these cultures were capsulated. The organisms of the second group consisting of 12 cultures, isolated from one quarter of a cow not included in the six mentioned above, were typical of *Escherichia freundii*. These cultures were non-capsulated.

Serological studies now in progress employing both slide and tube agglutination technics indicate that at least two serological types are present among the 76 cultures.

P54 Comparison of the Incidence and Severity of Mammary Edema of Cows Fed Roughages Alone or Roughages Plus Grain during the Dry Period. F. C. FOUNTAINE, D. B. PARISH AND F. W. ATKINSON, Kansas Agricultural Experiment Station.

Cows of the Ayrshire, Guernsey, Jersey and Holstein breeds were assigned alternately, within the breed, to two groups. So that they would be in a good state of flesh at time of turning dry, cows in group 1 were fed additional amounts of grain during the terminal 3 mo. of lactation. During the dry period their ration was limited to alfalfa hay *ad libitum*, plus atlas sorgo silage. Cows in group 2 were fed according to production during the terminal stages of lactation, and were fed alfalfa hay, sorgo silage and a 16% grain concentrate during the dry period.

Both prior to and subsequent to parturition there was no significant difference in the incidence and severity of mammary edema and congestion of cows in the two groups. The incidence of edema of varying degree of severity was 54% in the group fed grain during the dry period and 56% in the group that received only roughage.

P55 Diluting Bull Semen on the Basis of Numbers of Spermatozoa rather than by Volume. C. BRANTON, M. H. NEWSOM AND T. E. PATRICK, Louisiana State University.

This report deals with a study of diluting bull semen to contain 16, 12, 8, 6 and 4 million spermatozoa/ml. regardless of the numbers of spermatozoa in the undiluted semen samples. Fifty ejaculates from 10 bulls, 6 of them Jerseys, 2 Holsteins, and 2 Guernseys, were diluted with egg yolk-citrate-sulfanilamide and used in routine breeding. The experimental design employed

consisted of two 5 x 5 Latin squares. Results on the basis of 30 to 60 d. non-returns to 1,895 first services showed, 62.8, 64.0, 66.8, 58.0 and 63.5% non-returns for semen diluted to contain 16, 12, 8, 6 and 4 million spermatozoa/ml., respectively. When tested statistically, these fertility levels were not significantly different.

P56 Penicillin and Sulfanilamide in Semen Dilutors and Their Effect on Fertility of Semen from Relatively Fertile Bulls. J. P. MEYNER, New Jersey Agricultural Experiment Station.

To determine the effect of penicillin and sulfanilamide in combination on fertility of semen from bulls of relatively high fertility a 3 x 3 latin square was designed and replicated three times using a total of six Holstein and three Guernsey bulls. The following three egg yolk citrate dilutors were compared: (a) containing 1,000 units penicillin/ml. dilutor, (b) containing 3 mg. sulfanilamide/ml. dilutor, and (c) containing 1,000 units penicillin plus 3 mg. sulfanilamide per ml. dilutor. The data accumulated were for 27 semen samples from nine bulls and used on a total of 520 1st and 2nd service cows and were based on 60 to 90 d. non-returns. The mean percent fertility levels on the three dilutors were as follows: (a) 67.2%, (b) 74.4% and (c) 74.3%. An analysis of variance of the data indicated that there were no significant differences in these contrasted mean fertility levels.

P57 A Comparison of Penicillin, Streptomycin and Sulfanilamide for Improving the Fertility of Semen from Relatively Infertile Bulls. J. O. ALMQUIST, Pennsylvania State College.

In the present study seven relatively infertile bulls of the Western Pennsylvania Artificial Breeding Cooperative were used to compare yolk-citrate diluted semen and yolk-citrate diluted semen containing either 1,000 units of penicillin/ml., 1,000 units of streptomycin/ml., 1,000 units each of penicillin and streptomycin/ml. or 300 mg. % sulfanilamide. Each diluted semen sample served as its own control in that only half of the sample received one of the four treatments tested while the other half remained untreated. Two of the seven bulls were slaughtered soon after the beginning of the experiment because of extremely low fertility even though there was some indication of positive treatment response. The fertility of the diluted semen from the remaining five relatively infertile bulls showed a marked improvement when treated with penicillin, streptomycin or the combination. The control portions of 76 ejaculates were used for 1,814 inseminations and the treated portions for 1,807

inseminations. Based on 90 to 120 d. non-returns, fertility was increased 21.7 percentage units by penicillin, 25.9 percentage units by streptomycin and 21.3 percentage units by penicillin plus streptomycin. Sulfanilamide gave a slight decrease of 2.9 percentage units. The control semen averaged 38.9% non-returns.

P58 Fertility of Bull Semen Diluted from 1:100 to 1:300. E. L. WILLETT, American Foundation for the Study of Genetics.

Two small-scale and two large-scale experiments have been conducted to determine the fertility of bull semen diluted above 1:100. Non-returns were computed at an average of 75 d. after service. Yolk-citrate diluter was used in the first experiment and yolk-sulfanilamide-citrate in the others. Each semen collection, consisting of two or more ejaculates from a bull, was split three ways. One-third was diluted 1:100 and the other two portions were diluted at higher levels. The highest dilution studied was 1:300. The different dilution levels were rotated among different inseminator groups where the semen was used for breeding. In every experiment there was a downward trend in non-return rate with increase in rate of dilution, but no significant differences were obtained. When non-return percentages were plotted on a graph against numbers of spermatozoa per insemination, there appeared to be a curvilinear relationship with the rate of drop in non-returns increasing with decrease in sperm numbers. In the two large-scale trials with a total of 7,787 services from 54 collections from 18 bulls, for samples containing 6,000,000 sperm or more the regression coefficients were 0.43% and 0.52%—the decrease in non-return rate per million decrease in spermatozoa per insemination. For the samples containing less than 6,000,000 spermatozoa per insemination, the regression coefficients were 6.99% and 2.62%. The figure of 6,000,000 spermatozoa per insemination roughly represents, on the average, the dilution level of 1:200 when 1 ml. of semen is used per insemination.

P59 Buffered Whole Egg as a Nutrient Extender for Bovine Spermatozoa. H. O. DUNN and R. W. BRATTON, Cornell University.

A satisfactory bovine semen extender has been prepared by mixing one part of whole eggs, from which the chalazae have been removed and which have been thoroughly beaten in a Waring blender, with three parts of a buffer containing 1.93% sodium citrate dihydrate and 0.4% succinylsulfathiazole (sulfasuxidine).

The livability and fertility of spermatozoa stored at 5° C. in the citrate-sulfasuxidine-whole egg formula (1.9 CSSWE) and in the standard 2.97% citrate-sulfanilamide-yolk formula (2.9 CSAEY) were compared. Sixty-nine split ejaculates were extended to give approximately 16×10^6 live sperm/ml. extended semen. The estimated percentages of motile spermatozoa at 0 and 2 d. were 60 and 52 for the 2.9 CSAEY and 62 and 54 for the 1.9 CSSWE. The visibility of the spermatozoa and their rate of progressive motility was much greater in the latter. Based on 28 to 35 d. non-returns to 5,915 first-service cows, the percentage non-returns was 76.4 for the standard egg yolk formula and 74.3 for the whole egg formula. This difference is not statistically significant. Citrate-whole egg and citrate-sulfanilamide-whole egg, because of their high pH (7.8 to 7.9) were found to be spermicidal. The acidity of sulfasuxidine was found to be sufficient to alleviate this effect.

P60 The Fertility of Bovine Semen Cooled with and without the Addition of Citrate-sulfanilamide-yolk Extender. R. H. FOOTE AND R. W. BRATTON, Cornell University.

The cooling and extending procedures (a) semen cooled rapidly (from 30 to 5° C. in 5 min.) unextended, then extended 1:100; (b) semen cooled slowly (from 30 to 5° C. in 75 min.) unextended, then extended 1:100; (c) semen extended 1:100 and cooled rapidly; and (d) semen extended 1:100 and cooled slowly were compared using 11 split ejaculates extended with citrate-sulfanilamide-yolk. The mean percentage of motile spermatozoa during a 6-d. storage period for treatments a, b, c and d were 29, 47, 57 and 62, respectively. All treatments except c and d were significantly different from each other.

The fertility of semen cooled slowly with and without the addition of extender was determined on 64 ejaculates split two ways and used for insemination. When stored at 5° C. the estimated percentages of motile spermatozoa at 0 and 2 d. were 63 and 51 for the semen cooled in extender and 48 and 34 for the semen cooled without extender. The percentage non-returns on a 28 to 35 d. basis for 3,067 first services, and representing 32 of the 64 ejaculates, was 76 for the semen extended before cooling and 71 for the semen extended after cooling. The difference is statistically significant (<0.05 P).

P61 Relation of the Eosin-aniline Blue Staining Method to the Quality of Bull Semen. H. E. SHAFFER AND J. O. ALMQUIST, Pennsylvania State College.

Additional evidence that the eosin B-aniline blue staining mixture can be used to differentiate living and dead bull spermatozoa has been obtained. Spermatozoa rapidly lost their ability to remain unstained when undiluted semen was subjected to adverse conditions of temperature. The ability of the spermatozoa to remain unstained in the presence of the staining mixture did not appear to be dependent upon respiration, glycolysis or the presence of seminal plasma.

Field trials were conducted at the Nepa and First Pennsylvania Artificial Breeding Cooperatives to study the relationship between the percentages of unstained (living) sperm and fertility expressed on the basis of 90 d. non-returns. The study included a total of 197 ejaculates from 40 bulls. Each ejaculate was used to inseminate from 25 to 175 cows for a total of 10,344 services. A highly significant curvilinear regression was found between per cent 90-d. non-returns and per cent unstained sperm. The shape of the curve suggested that the staining method was of most value for detecting semen samples of relatively low quality. Under the conditions of this fertility study, however, it seemed to be of questionable value in predicting the potential fertilizing capacity of semen of relatively high quality.

P62 The Effect of Frequency of Collection upon Semen Production and Fertility of Dairy Bulls Used in Artificial Breeding. T. E. PATRICK, C. BRANTON AND M. H. NEWSOM, Louisiana State University.

This study was undertaken to determine the effect of various time intervals between collections upon semen production and fertility of dairy bulls. The duration of this experiment was 180 d. (three 60-d. periods). Two 3×3 latin squares were used with six bulls being subjected to the following treatments: I, one ejaculate every 4th day; II, two ejaculates every 8th day; and III, three ejaculates every 12th day. Volume, motility and disposal were recorded on each ejaculate. Methylene blue reduction time was determined on samples having a motility of 50% or better and a concentration above 900,000-000/ml. Ejaculates meeting certain quality standards were diluted with egg yolk-citrate-sulfanilamide and used in routine breeding.

Analyses of the data on semen characteristics showed no significant differences between treatments for volume per ejaculate, percentage motility, methylene blue reduction time and percentage of shippable ejaculates. Fertility results involving 2,794 first services calculated on 30 to 60-d. non-return bases were as follows: Treatment I, 69.4%; II, 68.3%; and III, 67.0% These dif-

ferences were not significant when tested statistically.

Data presented indicate that dairy bull semen can be collected once every fourth day with results equal to those obtained from less frequent service.

P63 Clipping as an Aid to Control of Cattle Lice. R. B. PRICE JR., W. C. PRIGGE, N. N. ALLEN AND R. J. DICKE, University of Wisconsin.

Twelve yearling dairy heifers, taken at random from a lot of 22, were clipped December 8. They were housed with the ten unclipped animals, running together in a large pen. When examined December 22, all of the unclipped heifers were infested lightly with lice, while no lice were found on the clipped animals. A second examination January 10 indicated an increase of lice on the long haired heifers, while the clipped animals remained free except for isolated lice found on two animals. On February 18, infestations had become fairly heavy on some of the unclipped heifers. Only very light infestations were found on any of the heifers which had been clipped, although their hair was well grown out. At that time, two of the previously clipped heifers were re-clipped and two of the long-haired animals were clipped. When examined 3 d. later, the freshly clipped animals were free of lice. A fifth long-haired heifer, heavily infested with lice, was clipped on one side only. When examined 3d. later, the clipped side was free of lice, while the unclipped area remained heavily infested. This was not the result of removal of the lice with the hair, as large numbers were found clinging to the base of the short hairs immediately after clipping.

No treatment for control of lice was applied to any of the heifers. Continued observations are being made through the early spring months. Those made to date indicate that clipping is a very definite aid in controlling cattle lice.

P64 The Effect of Methods of Milking, Methods of Cooling the Milk and Types of Barns on the Total Bacteria Count and Coliform Count. C. C. FLORA, P. M. REAVES AND C. W. HOLDAWAY, Virginia Agricultural Experiment Station.

Studies were made on cooling milk by two methods, pouring the uncooled milk into cans and setting the cans into a wet storage or cooling the milk over an aerator and cooling it before setting the cans into wet storage. An agitator circulated the water around the cans in the storage. The milk was not stirred. When the uncooled milk was placed in the wet storage, the bacterial count

increased at a somewhat greater rate during a 12-hr. storage period than when it was cooled over the aerator before placing in the wet storage. Similar trends were secured for the coliform count. In all cases the coliform count increased at a faster rate than the total count. The increase was very much greater in the uncooled milk.

Further studies compared milk produced when cows were housed and milked in a stanchion barn and milked with a standard type milking machine, when cows were housed loose and milked in a stanchion barn with standard type milking machine, when cows were housed in a tie-stall barn and milked with a combine type milker in a milking parlor, and when cows were housed loose and milked with a combine milker in a milking parlor. The milking parlor method gave lower total bacteria counts than milking with the standard type milking machine. Very little difference was found in the milk when cows were housed in the stanchion barn as compared to loose housing, both groups being milked in the stanchion barn.

P65 Some Observations on Recovery in Dairy Production in Western Europe. W. H. RIDDELL, University of Vermont.

The low point in dairy cow numbers in occupied countries and Switzerland occurred in 1944-5. For the Netherlands, this represented 75% of pre-war figures. Milk cow population in 1947 for eight countries (Belgium, Denmark, France, Netherlands, Norway, Sweden, Switzerland and United Kingdom) averaged 90% of 1934-8. Total milk production was approximately 80% due to limited import feed supplies and fairly widespread drought.

War-time reduction in livestock numbers for some occupied countries resulted in improvement in quality of dairy cattle and other livestock. In the Netherlands, where the decline was most severe, breed and livestock officials emphasized this improvement for all classes.

Percentage of cows in various forms of production testing has increased in post-war years. Except France, for which data were lacking, total cows tested in seven countries for 1947-8 were 2.5 million, approximating 28% of dairy cow population. Percentage range was from 8.5% for Belgium to 49.6% for Denmark.

Approximately one million cows were bred artificially in six countries for the same period, representing about 13% of dairy cow population. Percentage range was from 1.5% for Belgium to 33% for Denmark. Data were lacking for France, and Switzerland reported interest only in combating disease.

Dairy research was curtailed severely during war years in occupied countries.

P66 Feeding Value of Dehydrated Sweet Potatoes Fed Wet as Compared with Corn-soybean Silage for Lactating Cows. L. L. RUSOFF, B. J. BURCH, JR., J. B. FRYE, JR. AND G. D. MILLER, Louisiana State University.

It now is recognized that dehydrated sweet potatoes are approximately 90% as valuable as yellow corn meal as a source of carbohydrate in the grain ration. Using a latin square design, three groups of eight dairy cows each, consisting of five Holsteins and three Jerseys, were given a good grain mixture according to production. Approximately 8 lb. of alfalfa hay/cow/day and equal amounts on an air-dry basis of (a) corn-soybean silage, (b) dehydrated standard sweet potatoes wet with an equal weight of water or (c) dehydrated weevily-infested sweet potatoes wet with water were fed. There was no apparent significant difference in the milk production between the various groups on either silage or dehydrated sweet potatoes fed wet.

P67 Effect of Excess Concentrate Feed Consumption on Milk Production of Dairy Cows in Hawaii. L. A. HENKE, University of Hawaii.

Commercial dairymen in Hawaii often feed well above the requirements of the Morrison Standard. The general plan to feed 1 lb. of grain for each 3 lb. of milk produced is inadequate in Hawaii for two reasons. Roughages fed are coarse and of low nutrient content; the concentrate mixtures largely are based on two by-products with low nutrient content, pineapple bran and cane molasses, to which are added such protein supplements as may be needed.

The effect of excess concentrate feeding was studied in three trials using 22 cows. The concentrate mixture consisted of 25% cane molasses, 43% pineapple bran, 30% soybean oil meal and 1% each of bone meal and salt.

Cows when fed according to the Morrison Standard consumed 15.41 lb. of the concentrate ration to produce 24.06 lb. of 4 per cent fat-corrected milk. Cows fed excess concentrates consumed 19.76 lb. of the concentrate mixture to produce 25.41 lb. This shows a profit on the cost of the added concentrates of 2.13% when selling milk on the wholesale basis (18 cents) and 59.34% on the retail basis (28 cents).

P68 Influence of Various Udder Treatments Upon the Let-down of Milk. C. E. KNOOP

AND C. F. MONROE, Ohio Agricultural Experiment Station.

Ten milking trials were conducted with 11 cows to determine the influence of various pre-milking treatments on the rate of milk let-down. The following treatments have been studied: (a) no treatment, (b) dry hand massage, (c) the use of a strip cup, (d) cleaning the udders with a damp cloth, (e) cleaning the udders with a towel removed from hot water (120° F.), (f) the same treatments as e, followed by the use of a strip cup and (g) a thorough bathing of the udder in hot water, together with the use of a strip cup.

The effects of the above treatments upon the let-down of milk were measured by collecting the milk produced during the 1st and 2nd 45 sec. of the milking period in a specially designed milking unit (Surge). Data also were obtained on the total amounts of milk produced and the time required to milk each cow after the various treatments.

Results indicate the desirability of some preliminary treatment. Merely removing one or two streams of milk from each quarter did not appear entirely adequate for a complete let-down. A 10 sec. massage of the udder with a damp cloth was just as effective as a like treatment with heavy towels removed from hot water at 120° F.

P69 A Comparison of Milk Production between the Prepartum Milked Halves and the Non-prepartum Milked Halves of Bovine Udders. M. L. DAUDY AND C. B. KNOTT, Pennsylvania State College.

Twenty-two first-calf heifers of the five major dairy breeds and 13 Holstein cows have been used in a comparison of the effects of prepartum milking upon milk production, by pre-partum milking one-half of the udder and non-prepartum milking the other half.

A comparison for the first 30 d. postpartum of 15 first-lactation heifers, having apparently balanced udders, resulted in an average production of 529.4 lb. for the 15 premilked halves and 490.2 lb. for the 15 non-premilked halves.

Milk production of separate halves, obtained once every 15 d., has been used to calculate the total monthly production of 13 Holstein cows and three Holstein heifers for the first 7 mo. of their lactations. Only nine udders appeared balanced, of which the prepartum milked halves averaged 5,061 lb. milk and the non-prepartum milked halves, 4,780 lb. or an average difference of 280 lb. of milk in favor of the premilked halves.

P70 The Effect of In Vitro Treatments with Testosterone on the Oxygen Consumption of

Ejaculated Spermatozoa. F. N. BAKER, A. B. SCHULTZE AND H. P. DAVIS, University of Nebraska.

Samples of semen from nine bulls were selected at random, divided and diluted 1:4 in citrate diluter. One portion of each ejaculate served as a control, the other was treated with testosterone so that the final concentration of testosterone in the diluted semen was 2.6 mg./100 ml. Oxygen consumption of the treated and control semen was determined by the direct method of Warburg. In nearly every instance treatment resulted in a marked decrease in oxygen consumption. The average oxygen consumption in mm.³/hour for 2 ml. of diluted semen was 25.7 for the control samples and 20.5 for the treated samples.

P71 Complementary Effect of Acetylcholine and Thyroxine on O₂ Consumption of Bovine Semen. A. B. SCHULTZE, University of Nebraska.

The mean O₂ consumption of 15 semen samples with an original spermatozoan concentration of over 800,000 per mm.³ and less than 1,400,000 per mm.³ and treated with 7 to 10% thyroxine was 64.11 mm.³/hr. for the untreated portion and 69.97 mm.³/hr. for the treated portion, a highly significant difference.

The mean O₂ consumption of 15 semen samples with an original spermatozoan concentration of 800,000 per mm.³ or less and treated with 7 to 10% thyroxine was 51.71 mm.³/hr. for the untreated portion and 51.60 mm.³/hr. for the treated portion, a non-significant difference. When 0.0032% acetylcholine was added to bovine semen with a spermatozoan concentration of 800,000/mm.³ or less and the control portion consumed 46.05 mm.³ O₂/hr. and the treated portion 46.12 mm.³/hr. (means for 9 determinations), a non-significant difference.

When 0.0032% acetylcholine plus 10% D,L-thyroxine was added to semen with a spermatozoan concentration of 800,000/mm.³ or less the control portion consumed 51.44 mm.³ O₂/hr. and the treated portion 55.10 mm.³ O₂/hr. (means for 14 determinations), a highly significant difference.

P72 Recovery of the Fertilized Ovum from the Living Cow. A. E. DRACY, South Dakota State College, and W. E. PETERSEN, University of Minnesota.

A technic has been developed whereby the fertilized ovum can be flushed out of the uterus of the living cow without surgical intervention. The cow is inseminated when in estrus and the egg recovered on the 7th day. At this time it

descended the Fallopian tube and has not yet commenced nidation.

Instruments have been developed to facilitate entrance through the cervix. These consist of 5/16 inch stainless steel probe with a tight fitting cannula. The probe first is inserted through the cervix. This is accomplished by grasping the cervix by one hand in the rectum while the other hand is used for traction and manipulation of the probe. After penetrance of the probe the cannula easily may be slipped over the probe through the cervix and the latter is removed. A small Koreséal tube is passed through the cannula to the tip of the uterine horn on the side ovulation has taken place. Its course through the uterus may be guided manually from the rectum. Approximately 1 l. of flushing fluid is forced as rapidly as possible through the tube and the flushing caught in a separatory funnel from the cannula. Physiological saline has been used as the flushing fluid.

The material is permitted to stand in the separatory funnel for 15 to 20 min. when a few ml. of solution are withdrawn and observed under a 15 to 25 power dissecting microscope. The French type of separatory funnel has been found superior to the ordinary kind. The fertilized ovum gravitates to the bottom of physiological saline solution readily but often will adhere in mucous material that may stick to the walls of ordinary separatory funnels.

While there is great variation in the ease with which entrance through the cervix may be effected, in typical cases the whole procedure in flushing out the uterus need not exceed 10 min.

P73 Factors Affecting the Interval Between Parturition and Subsequent Estrus in Dairy Cattle. J. H. EDMONDSON, University of Missouri.

With the increased use of artificial insemination in breeding dairy cattle there have come many questions as to the length of time between parturition and the first estrus period. Through the detailed records kept on the Missouri Station herd, it has been possible to investigate this problem. The breeding records of 347 cows with 968 parturitions were studied.

Results showed that the average length of the interval from parturition to the first subsequent estrus period was 57 d. with a standard deviation of 28 d. There seems to be no relationship between the seasons of the year and the length of time from calving to the first estrus period after calving. The daily level of milk production did not appear to effect the interval between calving and occurrence of first estrus. A study of the effect of age on the length of the

interval from calving to first heat shows this interval becomes shorter with age until the 4th year is reached and increases in length for the 6th and 7th years when it decreases again. The interval between 1st, 2nd, 3rd and 4th calves, etc., followed a pattern similar to that of age; however, the similarity is not pronounced after the 4th or 5th calf.

P74 Comparison of pH Values of In Vivo and In Vitro Determinations on Bovine Vaginal-Cervical Mucus. D. B. ROARK AND H. A. HERMAN, University of Missouri.

In vivo and *in vitro* pH measurements on vaginal-cervical mucus were made simultaneously on ten cows during various phases of estrus. The pH determinations were made with a Beckman pH meter using a small glass electrode for the *in vitro* and a special silver-silver chloride electrode for the *in vivo*. Thirty paired observations showed, in each case, that *in vivo* pH was more acid than *in vitro*; however, the magnitude of the differences was inconsistent. The *in vivo* pH values averaged 6.57 and ranged from 0.40 to 1.33 (average 0.88) lower than *in vitro*. The differences between *in vivo* and *in vitro* pH values were significant at the 1% level.

It is believed that these differences in pH values are not due to losses of CO₂ from the results of an experiment in which the pH of ten paired samples (draining and aspirated) of mucus was determined within 1 min. after *in vivo* determinations. A slow drop occurred in the pH of *in vitro* samples during the first 20 min. following collection, averaging 0.16 for draining samples

and 0.19 for aspirated samples. The *t*-test showed no significant differences between aspirated and draining samples. Earlier workers found that a film is formed about an electrode in contact with moist tissue and that a difference in electrical potential may exist. Perhaps this was a contributing factor to the observed differences between *in vivo* and *in vitro* pH values of this experiment.

Further studies of the cyclic variations in the physical and chemical properties of bovine mucus are in progress and may enhance our knowledge of the factors affecting breeding efficiency where artificial insemination is practiced under field conditions.

P75 The Interrelationship of Age and Season on Bull Fertility. T. M. LUDWICK, D. S. RUDRAIAH, J. ROSENBERGER AND F. ELY, Ohio Agricultural Experiment Station.

Investigations were made on data from the two artificial breeding associations of Ohio and include approximately 70,000 1st services from the Central Association and 120,000 1st and 2nd services from the Northern Association. The data cover a period of 2.5 yr. for Central and 5 yr. for Northern. Records were summarized by age groups of bulls, seasons and breeds. Only bulls which had been used for breeding more than 1,000 cows/yr. were included in the study.

Variation in conception (measured by 60 to 90-d. non-returns) as influenced by season or age of bull is not highly significant. Breed differences are not influenced greatly by age variations.

Some significant differences between breeds may exist as influenced by seasonal variations.

EXTENSION SECTION

E1 Suggested Revisions in the D.H.I.A. Herd Record Book. J. F. KENDRICK, Bureau of Dairy Industry, USDA.

A discussion of possible revisions of the D.H.I.A. forms that they may more adequately serve the needs of the D.H.I.A. programs operating in the various states is presented.

E2 Comparison of D.H.I.A. Computing Tables. C. R. GEARHART, Pennsylvania State College.

The object of this study is to make possible the use of simplified computing tables to save time for D.H.I.A. supervisors and to reduce the number of mistakes made in those associations which do not have calculators.

2251 monthly records were calculated on 109 cows. These were distributed as follows: (a) Three consecutive years of a RH herd averaging over 400 lb. fat. (b) Three consecutive years of a RG herd averaging over 400 lb. fat. (c) Four

consecutive years of a P&GrG herd averaging less than 350 lb. fat. (d) Three consecutive years of a RJ herd averaging over 400 lb. fat. (e) A few lifetime records on cows having from three to six lactations.

Individual records were calculated for each cow each month. In addition to the regular D.H.I.A. calculations, three additional calculations were made of these same records by using three different modified computing tables. All monthly records were then placed on I.B.M. cards and comparisons of the various types of tables were made by the machine.

The results of this study show: (a) The use of simplified computing tables will save a supervisor much pencil work. (b) Multiplication mistakes will be reduced. (c) Part of the addition will be simplified. (d) Work will be easier for supervisor, thus there will tend to be fewer changes of supervisors. (e) Computing tables will not help associations with calculators.

E3 Progress Report on Use of I.B.M. Machines in Processing D.H.I.A. Records. H. C. GILMORE, Pennsylvania State College.

The object of this study is to determine whether I.B.M. machines can be used to advantage in processing D.H.I.A. records. Some of the factors involved are saving time to the supervisor, accuracy of records and having the information in a more usable form. Temporary forms have been drawn up and plans have been developed to study the use of these machines in one full association and a few herds in different sections of the state for a period of 1 year on a trial basis. This will be in addition to the regular D.H.I.A. work, so that a comparison of the two systems and their relative merits can be determined.

Identification appears to be one of the big problems at the present time. Ear tag numbers present a problem as well as the length of some registered names because the number of columns on the cards is limited. If some of the records could be shortened it would simplify the use of punched cards because of the lack of space on the cards.

Along with this procedure, a study is being made of new methods of sampling in the field and it would seem that more use could be made of D.H.I.A. records from an educational viewpoint if the information were recorded on punched cards.

E4 Use of I.B.M. Equipment for More Efficient Processing of BDI 718 Reports. R. ALBRECHTSEN, Cornell University.

With the renewed expansion of D.H.I.A. has come the problem of handling the increased volume of data efficiently. This applies particularly to BDI 718 lactation reports. These reports are the key information to evaluating the artificial insemination program's effect on herd improvement, sire proving and herd analysis. The usual methods for handling this data require a great deal of labor which cannot be provided as the BDI 718 reports increase. Recourse to modern methods for handling data on I.B.M. equipment seems to be a feasible solution.

The processing of data such as is reported on BDI 718 cards has little precedence in the experience of I.B.M. fieldmen. A system of transferring data to I.B.M. cards with subsequent sortings, machine calculations of mature equivalents and final tabulation of summarized results call for a complicated series of I.B.M. processes. However, once the system is mastered, its versatility and efficiency becomes apparent. Regular personnel is trained in these new methods. The possibilities of I.B.M. processing of BDI 718 records are not yet fully apparent.

E5 Centering Date Versus Calendar Month for Computing Dairy Cow Production Records. R. MORRISON AND R. E. ERE, Washington State College.

The present method of calculating D.H.I.A. records is on a centering date basis. The involved procedure for calculating credit due a cow is confusing. Many errors in calculating centering dates have been found in D.H.I.A. record books. These errors, the extra time required and the fact that dairymen know when the tester is coming make this system undesirable. Calculating records on a calendar month basis would correct these errors. However, the accuracy of the measure of production on a calendar month basis as compared to the centering date basis is unknown.

To study this problem, the Holstein-Friesian Association has made available 24 lactation records, each 365 d. 4 ×, and each milking being weighed and tested. Ten cows in the college herd were milked 305 d. 3 ×, with each milking being weighed and tested. Also nine cows in the college herd were milked 305 d. 2 ×, with each milking being weighed and tested. Eight periods were selected in each month and production for both calendar method and centering date were computed. This production was compared to the actual production. A statistical analysis of these records was presented.

E6 Extension Education on Milking Machine Operation. I. E. PARKIN, Pennsylvania State College.

Pennsylvania dairy farmers have been sold milking machines without receiving the fundamental instructions required to operate them efficiently and to keep them clean. An extension program launched in 1943 on managed milking has branched out to include proper machine operation, installation and care of milking machines and the cleaning of the vacuum line. This program has been accomplished by demonstrations on managed milking, county-wide milking machine clinics and demonstrations of cleaning vacuum lines. These meetings have been requested by county agents, plant field men, sanitarians, dairy farmers and milking machine dealers. The cooperation of milk plant personnel, milking machine dealers, dairy cooperatives and farmers has been extremely gratifying. Apparently farmers are interested in the program because attendance has been more than satisfying. The subject matter presented included installation and care of the milking machine, washing and sanitizing milking machines, cleaning vacuum lines, managed milking, milk secretion, mastitis prevention and herd management. The

aims of this program are better herd management and better quality milk.

E7 Development of a Successful Integrated Dairy Program. E. C. SCHEIDENHELM, Rutgers University.

New Jersey's agriculture extension service has five major commodity groups. They are dairy, poultry, fruits, vegetables, and ornamental horticulture and home grounds. Contributions from other subject matter specialists to the dairy project results in a complete integrated dairy extension program. The subject matter specialists whose programs are included in the dairy program in New Jersey are agricultural engineering, farm crops, farm forestry, farm management, marketing, soils, soil conservation and human nutrition.

The steps followed in the development of the program were: (a) Conference of all specialists to discuss how their programs could be integrated with the dairy project. This meeting resulted in developing a "long-time dairy extension program." (b) Two or more conferences during each year to plan a series of integrated dairy institute meetings for the winter months. These are all-day meetings. (c) Additional meetings as requested by agents where coordinated teaching of two or more specialists is needed.

The dairy specialists carry on most of the work with reference to their sub-projects themselves. This also is true for the specialists who contribute to the integrated dairy program.

E8 The Michigan Program of Brucellosis Control in Cattle. R. E. HORWOOD, Michigan State College.

Part I of this paper reviews the progress of brucellosis control program in Michigan since 1930 and the accomplishments of the state brucellosis committee which include: (a) making available three general plans of testing on a herd or area basis; (b) enforcing the exhibition law for livestock; (c) returning copies of officially reported vaccinations to livestock owner; (d) reduced the time of returning results of tests to owner; (e) made possible the use of local veterinarians in area work; (f) prompt retests on a herd and area basis; (g) an extra bang's test at several state breed association sales; (h) secured the cooperation of government agencies and farmers associations; and (i) developed an educational program that has been carried out by the Extension Service to each county in the state and that resulted in all counties requesting an area test.

Part II reports on the use of *Brucella* M vaccine in Michigan since 1947 and states that: (a)

M vaccine in infected herds appears promising. In general it seems to stop the spread of infection effectively. (b) It does not produce long-lasting blood reactions in non-exposed animals. (c) A very small number of reactor animals show a significant decrease in blood reaction, but it is not advised for reactor cattle. (d) In accredited herds that desire to vaccinate only M vaccine may be used. (e) For adult vaccination, advise only M vaccine. (f) No evidence has been observed that M vaccine produces infection.

E9 4-H Show Programs as Developed in Mississippi. L. A. HIGGINS, Mississippi State College.

This paper discusses the limitations of the 1, 2, 3 system of placing cattle in 4-H shows and reviews the development, since 1940, of a group method of judging and placing entries of 4-H cattle. Briefly, the system developed is as follows:

The groupings were set up as nearly as was practical on the American Jersey Cattle Club's system of official herd classification. This was done to educate Mississippi farm youth on the principles of herd classification. Group terms were different, though perhaps as significant as are the official terms. They are: Superior, Very Desirable, Desirable, Medium, Fair and Undesirable. These various groupings carry the same score card rating as the corresponding rating in official classifications. The greatest difference is in the fact that under-producing ages are classified in this show system.

The "Superior" rating carries a purple ribbon. A female must have dropped her second calf and score 90 or more to qualify. "Very Desirable" ribbon award is blue. "Desirable" color is red and "Medium" is white. Neither money nor ribbon awards are given on animals which rate under medium in district and state shows. Judges are asked to follow actual score ratings as closely as possible, without actually scoring the animal point by point, and to place the top two to five animals of the "Very Desirable" group in 1, 2, 3 order, depending on the number permitted to show in the respective open show lots, also for championship competition. Each animal in a respective group receives the same amount of premium money. Junior owned bulls generally are permitted to show in the open-class shows only.

E10 Training 4-H Dairy Project Leaders. E. T. ITSCHNER, M. J. REGAN AND W. H. CLONINGER, University of Missouri.

This paper emphasizes the value of the demonstration method of teaching in 4-H club work and

describes the development of a type of leader training meeting at which project leaders are trained to perform method demonstrations which these leaders in turn present at 4-H club meetings. Leader training meetings are held on farms where facilities for group work are good and where there is sufficient livestock of various ages to permit leaders to practice after seeing the demonstrations. Demonstrations given at leader training meetings include casting, dehorning, tattooing, drenching, fitting, fast milking, cleaning and care of utensils, removing extra teats, trimming feet, estimating weight, keeping milk production records, preparing registration and transfer forms, and treatments for ringworm, lice and warbles. Other subjects discussed include mastitis, calf feeding and calf quarters. Since this method of leader training has been used, 4-H club membership in dairy projects has increased from 672 to 2605.

E11 Analysis of Production Records of the Daughters of Sires Used in the New York Artificial Insemination Program. RAYMOND ALBRECHTSEN, Cornell University.

The D.H.I.A. records made by progeny of sires used in artificial insemination are providing unexcelled data to measure various phases of dairy production within a state. Primarily, these records serve to measure the influence of carefully selected sires on the D.H.I.A. segment of the dairy industry. Since this D.H.I.A. segment is regarded as a basic dairy demonstration, the situations observed can be used as teaching materials to encourage adoption of improved dairy practices.

The records used in this study are those reported to the Dairy Record Office at Cornell on the standard report card, BDI 718. These data were transferred to I.B.M. cards and were sorted, summarized and tabulated to give the following studies: (a) A study by levels of production of the mates of proved and analyzed young sires shows the possibilities for herd improvement on cows that average up to 420 lb. of fat. (b) A study by levels of production of daughters of sires used shows the variability of a cow population in support of good sires. Many high-producing dams do not have satisfactory daughters. (c) A study of regional differences in response to the use of the same sires shows production apparently is better in certain areas of the state. (d) A study of differences between herds in the effect of the use of sires in artificial insemination reveals that previous breeding programs affect the response to the AB sires. (e) Study of reproof of natural service proved sires and their performance in relation to proved sire performance in general shows that reproof in artificial insemina-

tion will establish more firmly a bull's genetic value. (f) Study of analyzed sires proved in artificial insemination in relation to proved sires in general provides a check on a method of selecting young sires.

As further data accumulate, it becomes important to re-check all studies to ascertain changes that may affect the conclusions drawn from these data. These AB daughters will provide an unparalleled opportunity to check the affect of proved sires, analyzed sires, environmental influences and the genetic qualities of the mates of the bulls as well as the bulls themselves.

E12 A Different Slant on Sire Selection. W. E. WASHBON, West Virginia University.

Sire selection methods in common use today hold no promise for consistently selecting bulls that offer better than six out of ten chances for improving production at a profitable level. Artificial breeding cooperatives and individual breeders as well have great need for a method of selecting young sires that will reduce their chances of getting a really poor bull and greatly increase the chances for getting an outstandingly good one. The results received by dairymen who have selected 25,000 D.H.I.A. proved bulls should indicate how to select uniformly herd-improving bulls.

A study of the results received by 1,500 Holstein breeders who selected sons of D.H.I.A. proved bulls reveals: (a) Ten or more comparisons are needed for sire selection purposes. (b) The greater the proof increase, the better the results. (c) The higher the level of production in the sire's proof, the better the results. Outstanding results are found when proof is above a 475 lb. fat level. (d) Sons of minus proved bulls offer less than a 50% chance for herd improvement regardless of the number of comparisons, the amount of decrease in production or the butterfat production level of the daughters.

The results received by 916 Holstein breeders who selected grandsons of 33 famous sires indicate: (a) Sires with uniformly outstanding proved sons have uniformly outstanding proved grandsons. (b) Those sires having 65% or more of sons plus proved, whose sons' daughters averaged at least 430 lb. of fat, an increase of at least 20 lb. over their dams, seemed to offer outstanding possibilities in sire selection.

The possibility of analyzing sire pedigrees for actual proof of transmitting ability, not only as immediate descendants of proved bulls but as descendants of outstanding bull families as well, offers real opportunity. The combination supplements the assurances of one method with the assurances of the other.

FORTY-FOURTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

P. R. ELLSWORTH, *Secretary-Treasurer*

The American Dairy Science Association assembled in the Main Ballroom of the Coffman Memorial Union, Minneapolis Campus, University of Minnesota, Minneapolis, Minnesota on June 21st, at 9:30 a.m. J. B. Fitch, local chairman, introduced Dean T. C. Blegen, who gave the welcoming address as official representative of President J. L. Morrill, University of Minnesota.

Association President W. E. Petersen was introduced next and gave the following address:

THE PRESIDENT'S MESSAGE

The American Dairy Science Association has for its objective the promotion of the entire dairy industry through research, teaching and extension with the firm conviction that by sound promotion of this great segment of American agriculture, the best interests of humanity are served. A sound agriculture that produces efficiently an abundance of food of a great variety is the most important single requisite for a high living standard. Without a diet that is adequate in promoting the best well being and of satisfying the palate demand, a people will not be happy, and if the cost of such a diet is too great there will be little left to acquire the other essentials for high standards of living. Everyone, therefore, whether urban or rural dweller, should have an enormous interest in agricultural promotion. Rightly, agricultural research should be supported by general taxes because all benefit from its contribution in providing more abundant food more efficiently produced.

In this connection, only those farmers who adopt new technological advances early will benefit in greater profit. As such technological advances that increase efficiency are universally adopted, the results reflect in a lower price to the consumer because of the highly competitive nature of agriculture.

The dairy enterprise is the largest of all farm enterprises in America. It is responsible for about a fifth of the total farm income and supplies about a fifth of the American food. This enterprise contributes enormously to an improved quality of the diet and therefore the general well being of the people. Milk and other dairy products hold pre-eminent positions among the nutrition specialists as the best foods for not only children but adults as well.

Dairy products serve well in satisfying the palate appeal as well as a source of good nutrition. Milk as such or any of the many products such as cream, whipped cream, ice cream and the numerous varieties of cheeses have enormous appetite appeal.

Dairying serves humanity not only in these particulars but plays a very important part in assuring a permanent, efficient agriculture. Much of our agricultural land is unsuited to tillage because of erosion hazards and should be kept in grass. Other land has been depleted in fertility because of over cropping and bad soil management practices needing rejuvenation calling again for growing of grass and legumes. Grasses and legumes that are needed in the preservation and improvement of soils cannot be consumed as such by human beings. Our digestive tracts are not constructed for such materials but are well suited to animal products produced for them.

The cow has been designed for this job of converting roughage into milk and meat. She is endowed with a structure—the rumen—that is specifically designed to handle bulky rough feeds. This, coupled with the fact that she has an extraordinarily highly efficient complex factory, the mammary gland, puts her in a class by herself in the economy of food production. No other animal has the potentialities to serve man as effectively or as efficiently as the dairy cow. We in the field of dairy science, whether research worker, teacher or extension worker, have every reason to feel proud of our field of endeavor as it serves humanity so well.

Let us now in all humility take stock of our accomplishments and then of some problems as we face the future. We cannot, of course, claim credit for all the advances that have been made in the dairy industry, but I am certain that a fair share of the improvements made can be credited to the people who have carried on research, teaching and extension work in dairying. During the last 50 years the average production per cow has doubled. The quality of dairy products have been greatly improved, new products have been developed and perhaps most significantly the receptivity of both dairy farmers and dairy manufacturers for information has been enormously improved. At the time this organization was formed, it was difficult to get an audience, while now the demands for new information cannot be filled.

The factors that have contributed to the improved situation with relation to the dairy enterprise are legion and cannot be dealt with in the limited time available. Better to give the remainder of the time to our problems as they face us.

The dairy industry cannot progress any faster or further than the quality of the men in this organization will permit. Our first and most important problem therefore, is that of obtaining the right kind of men and giving them the proper training. This problem has always been with us. Interestingly enough, the very first meeting of this Association in 1906 stressed this point by having inserted in the published proceedings the following: "Emphasis was placed on the demand for more and better trained men in dairy work and raising the standard of dairy instruction." With the enormous technological development in dairying and allied fields since that time, the problem of training men is greatly increased as is the need for better trained people.

Every segment of the dairy industry is very much concerned with research and teaching in its larger sense; as a matter of fact its progress is pretty much dependent on these two. This Association began as the National Association of Dairy Instructors and Investigators and since was changed to the American Dairy Science Association. While recognition of teaching has been dropped from its title, it is still of the highest importance for the furthering of the industry whether on the undergraduate or graduate level. Let us never under-rate good teaching. Our technological knowledge is of little avail without its spread to those that shall use it and our research workers must also be produced by the teacher.

This Association has always been aware of the teacher and has had committees from time to time for studying the problems incident to the worker in the classroom. With all that, the good teacher or trainer of men does not receive the recognition he merits. We have recognition and awards for those who excel in research, but we have never found a way to recognize the good teacher upon whom we are so dependent. It would be an added stimulus to good teaching if we could find some way to give recognition to the good teacher. One way, of course, that is not under the control of this Association, is giving him a salary more nearly commensurate with his contribution.

The qualifications of a good teacher in dairy husbandry are no different from those of teachers in other fields. He must be well informed and keep up with all new developments in the field. He must be enthusiastic about the subject and have the capacity to radiate that enthusiasm to his students. He must have a good perspective of the

whole and have a sound philosophy—not disregarding the old because it is old; likewise not to adhere to the old because of tradition. The new, likewise, must not be accepted with blind enthusiasm because it is new nor rejected simply because it is new. A healthy critical view must be taken by the teacher which will reflect quickly on the student. These are but a few of the qualifications of a good teacher.

The extension worker is a teacher and what has just been said applies to him equally well. The extension worker is a very important wheel in the complex educational machinery. It is he who is largely responsible for new information and practices getting into the hands of the ultimate user. The idea once entertained by some that the dairy extension worker should be a practical man with a pleasing personality, a gladhand man who had a few recipes that could be forcibly given with a good selection of funny stories is no longer tolerable. He, of course, must have personality and must know enough about the practical side to gain confidence, but to do the proper job, he must in addition be above all else well-informed on all aspects of dairying and its inter-relationship with other agricultural enterprises. This holds equally well for extension in production and manufacturing.

There is nothing that will depreciate the confidence in the extension worker more than an ignorance on his part of the technological knowledge on the subject. The farmer of today is not satisfied with a recipe. He wants to know the facts—he wants evidence and he is entitled to it. There is a marked change in the extension workers clients as contrasted to a generation ago. The dairy farmer is a much better informed individual. Here the agricultural press has contributed enormously and also furnished evidence of the evolution in the level of education. Note the technical nature of our agricultural press of today as contrasted to that of a generation ago.

For the extension worker to keep up on the new technological developments in the dairy field is no easy task. Too often administrators consider that he works only when he is out in the field and neither time nor facilities are given him for the continuous education that he must carry on to be the most effective. Our JOURNAL OF DAIRY SCIENCE is the most important source of new information for the extension worker as well as others. The original papers, if studied, will give a fairly comprehensive picture of developments in America. The review or abstract section, as it has been improved by our capable editor, is a splendid source of other pertinent dairy material. In addition, frequent conferences between the extension workers and the resident staffs are of mutual benefits to both.

Now we come to the research worker—the one who is responsible for the new technological developments that so often upset the mental equilibrium of dairy folks and seemingly keep them in a constant stage of uncertainty, for no sooner has one worked out what appears a sound plan than out comes a bomb-shell, a new discovery that disrupts everything. Partly because of this disturbance he creates in what might otherwise be calm water, and partly because of the inertia of tradition worshippers, some people have suggested that a moratorium be declared on research work. They use as an argument that our technological knowledge is much in excess of that used in general practice and that we need a rest from new knowledge so practice can catch up with knowledge.

There are several faults with this line of reasoning. One is that practice never catches up with knowledge. If it did, we would have reached perfection and that is too much to expect even from dairy folk. A second and more important point is that the greater the backlog of knowledge is, the more rapid will be the adoption in practice. Rather than too great a backlog of technological knowledge, we have entirely too little for the most rapid improvement in the practice. Due to curtailment of research during the war and concomitant cessation of training of research personnel, both the quantity and the quality of dairy research has suffered setbacks that will require still some time before recovery is made.

There are several reasons why a large backlog of scientific information is needed. One is the sheer pressure that is created by the mass of the information. This seems to be a physical phenomenon similar to hydrostatic pressure in which the velocity and extent of the spread of the technological knowledge is directly proportional to the head pressure. Another and very important fact is that a few of the most progressive adopt new technological knowledge as rapidly as it is advanced. The impact of these progressive individuals, although often few in number, upon the whole is beyond the realm of accurate evaluation. It is our duty to see that a constant and ever increasing flow of new information will reach them.

The selection and training of the research worker is one of the great and difficult responsibilities that befall many of us. Selection of the right kind of person is the first and by no means the least of these responsibilities. Only relatively few have the inherent potentialities to become good research workers. The desirable research worker must have an abundant amount of enthusiasm, an infinite capacity to work, the patience to wait, sometimes for long, for results; he must have imagination, the ability to question, the power to generalize and the capacity to apply.

Knowing the often meager material reward for services rendered, our candidate for training in research needs to be possessed of a such a temperament that he will be animated by the professional spirit which is one of service.

When we are lucky enough to discover such an individual, the second major responsibility begins—that of training. The type of training needed today is vastly different from that which was satisfactory a generation back. There was a time when all that one needed was the ability to apply a certain test pattern or formula. It was work of a nature to get the answer to practical problems as quickly and cheaply as possible. Typical of that type of investigation is the double reversal feeding experiments in which the relative merits of different feeds were determined. This type of investigation is not *passé* as much useful information is still to be obtained from its judicious application, but the work that will yield information of the greatest value to the dairy industry, in all areas, is fundamental research. The discovery that one feed is superior to another in milk production is of value, but of much greater value is to know in all details as to why it is better. Likewise, it is well to know that one procedure produces a better quality of milk powder, but it is infinitely more valuable to know why this is so. By knowing all of the fundamentals operative in a given area, a clearer picture of that area is possible which not only helps solve any new problems that may arise but is essential for suggesting new and better ways or methods.

To carry on modern dairy research requires special training. In an area with as many ramifications and as complex as that represented by this group training in many different disciplines are needed. No one can any longer prepare himself for the entire area of dairy science so he must specialize to a certain degree. Therefore, detail patterns will vary for individuals. There are some broad generalizations, however, that can be laid down. None of the physical or biological sciences is alien to dairy science. Botany, zoology, bacteriology, physics, chemistry, nutrition, physiology, anatomy, genetics and others have a direct bearing upon various areas in dairy science. It is not possible for anyone to obtain complete mastery of all of these specialties, but sufficient training in each of them so as to have a general grasp of their import is essential. This should be followed by mastery of one or more of them that are to be used directly in research work.

The general knowledge should be obtained in the undergraduate level. One of the major handicaps we encounter in the training of research workers is that candidates do not make up their minds to pursue graduate work, for the most part, until late in their undergraduate life or after hav-

ing completed a course in Agriculture. These people are not as well prepared for graduate work as would be those who could use their entire undergraduate time for preparation for graduate work. The ideal time to begin training for research work is at matriculation in college. If selection could be made at that time, an undergraduate course can be outlined that would not fit the student for a job at the end of the four year course because he would have very little, if any, of practical courses; but he would be fitted to really begin graduate work.

What should such an undergraduate course consist of? Stress would be placed upon mathematics, chemistry, biology, physics and of course a goodly portion should be given over to cultural courses. At the end of the four years, he should be culturally developed and have a foundation upon which to build his graduate education. Up to this point, it is unessential to have any special training in dairy technology. This can far easier be made up in graduate work than can deficiencies in fundamental work such as chemistry, mathematics, etc. Every effort should be made to ascertain as early as possible those who have the qualifications and the inclination to pursue graduate work that the undergraduate program may be better suited for graduate work.

In graduate training for research, an absolute requirement is thorough training in dairy science not only in the area of specialization but a comprehensive knowledge and understanding of the area outside of the field of specialization should be had that a sound perspective of the whole may be formed. But that is not enough. Modern dairy research that will most benefit the industry, demands research workers that have intensive fundamental training in one or more additional basic sciences. For dairy production physiology, biochemistry, nutrition, genetics and others not only are essential in their contribution to dairy research, but dairy research also offers great possibilities of making valuable fundamental contributions to these areas of learning. In dairy manufacturing, dairy bacteriology, dairy engineering or dairy economics, the need for fundamental training in chemistry, bacteriology, physics, engineering and economics are equally essential. Because of the enormous breadth of dairy research, no one can hope to master completely all of the basic knowledge essential to all dairy research, and therefore must make a careful choice of the area to which special attention must be given.

If the foregoing analysis of the needs in training research men are acceptable, it becomes obvious that for many research problems no one individual can acquire sufficient knowledge that by himself he can carry on the most effective work. This

means that more and more of dairy research must be carried on cooperatively between different specialty departments. Singularly, enough such cooperative projects are the more numerous and more satisfactory where the dairy science research partner has had training in the area of learning represented by the other cooperator. As one would expect, misunderstanding and disagreements arise mostly in cooperative projects when no such training has been had.

It will take time before we in dairy science will have a sufficient number of properly trained men to supply the need. We have quite a number of excellently trained dairy scientists, but we need more. For those who are skeptical about their existence look over the character of the papers listed in our programs for this and other meetings of this association. For an industry that holds the importance in the total economy and welfare of the country that is represented by this Association, we need many more men of the quality and training represented by our best research people.

Before leaving the question of research in dairy husbandry, let comment be made about giving opportunity to those who are well trained to make the most use of their abilities. Recognition must be made of the problems of the administrators with our project system which must be carried on and the lack of adequate funds to initiate and carry on special projects. As a result of this situation, the young research worker too often is made to fit in on projects where he has no opportunity to exercise his ability. Often his time is assigned to work for which he has no liking or special training—all of which is frustrating. To stimulate the most rapid development of the individual, it is essential that as much freedom of action be given as possible. The ideal situation for the right kind of a man is to furnish him a laboratory, with the necessary equipment and finances, and let him do whatever he wants.

Chairman Fitch then introduced Ancel Keys, Director of Laboratory of Physiological Hygiene, University of Minnesota who spoke on the subject of Cholesterol and the Problem of Ageing.

The following is an abstract of this talk:

Progress in preserving life and health in youth accentuates the medical problems of later life. In the United States the outstanding necessity of research on the ageing process is made clear by inspection of the vital statistics. Disease of the cardiovascular system is far and away the greatest killer now that so much has been accomplished in controlling tuberculosis and the diseases of childhood. And the largest part of cardiovascular deaths is related to the ageing process—high blood pressure, hardening of the arteries, coronary occlusion, "strokes."

We cannot hope to prevent ageing but perhaps we may attempt to delay or control it so the heart and blood vessels do not give way before their time or while the rest of the mind and body is relatively young.

The central feature of ageing in the cardiovascular system seems to be arteriosclerosis, or hardening of the arteries, which begins with an accumulation of fatty materials within the blood vessel walls and ends with a vascular tube either so reduced in bore that it cannot carry enough blood or so brittle that it bursts. Finally, the artery is full of a deposit of insoluble calcium salts but this seems to be a secondary consequence of the primary accumulation of lipoids in the wall. In a very real sense, then, one of the major problems of ageing is why, and how, this lipid deposit comes about.

The lipid deposit itself is peculiar in that it is made up of a large percentage of cholesterol, a lipid compound which is very peculiar to animals and is chemically related to other important substances, including some of the hormones. If cholesterol is not the "cause" of arteriosclerosis, it is at least importantly involved. In some animals administration of cholesterol can produce arteriosclerosis. Certain diseases, like diabetes and myxedema (thyroid deficiency), are notable in that the patients often have much cholesterol in the blood and tend to early and severe arteriosclerosis.

Recent arguments suggest that cholesterol obtained in the diet may promote arteriosclerosis. Dietary cholesterol comes from eggs, dairy products and meats—foods we generally consider to be very good nutritionally. Can it be that, as we improve the diet in general, we automatically increase the hazard of arteriosclerosis? The main arguments are: 1) Feeding large amounts of cholesterol to chickens and rabbits produces high blood cholesterol and arteriosclerosis. But these species ordinarily never have cholesterol in the diet and are scarcely comparable to carnivorous animals which are much more resistant to cholesterol feeding. 2) As our diet has improved to include more cholesterol-containing foods, the mortality from cardiovascular disease has increased. But this may be largely a result of an older population and better methods of diagnosis and recording. 3) It is claimed that the incidence of arteriosclerosis in different countries is inversely related to the cholesterol (and fat) content of the diet. But the data for this conclusion are as yet totally inadequate and are mainly only impressions from visitors to the Far East. 4) A low cholesterol diet reduces the blood cholesterol. But, though this is true with extremely low-fat diets, it is not proved with any lesser degree of restriction and

as to whether cholesterol or total fat, or both, are important is not known.

Recent work at the Laboratory of Physiological Hygiene has shown that the blood cholesterol increases with age from adolescence to the middle fifties; thereafter the values tend to decline. This suggests that, before the deposition of cholesterol in the arteries, there is a change in the metabolism of cholesterol independent of diet. Among men of the same age, the fatter men tend to have higher blood cholesterol concentrations. This suggests what we already know from vital statistics, that is overeating is a serious nutritional fault. Finally, a study of the habitual diets of 500 men who were also investigated with regard to blood cholesterol, showed no relation at all between dietary cholesterol and the amount of this substance in the blood. Special experiments with meals containing very large amounts of cholesterol confirmed this conclusion that, within wide limits, the amount of cholesterol in the diet and that in the blood are unrelated.

While these findings make us feel more at ease while eating our bacon and eggs and drinking milk at breakfast, the problem of cholesterol and arteriosclerosis is still present in all its mystery. We have merely made it clear that the body itself regulates its own content of cholesterol. We have still to discover how this is achieved and what there is in getting older and fatter that has such disastrous consequences. The Laboratory of Physiological Hygiene will continue to study these questions as a part of its long-range study on cardiovascular degeneration.

At a general session of the Association held on the Minneapolis Campus, June 22 at 4:15 p.m., W. W. Spink, Professor of Medicine at the University of Minnesota delivered an address on "Brucellosis in Man."

HUMAN BRUCELLOSIS

(Abstract)

Brucellosis may be caused by any one species of *Brucella*, namely, *Br. abortus*, *Br. melitensis* and *Br. suis*. The natural reservoir of this disease resides in domestic animals, particularly in cattle, hogs, and goats. The disease is very rarely transmitted from human to human. Man acquires the disease either through direct or indirect contact with infected animals.

A recent study in collaboration with the Laboratories of the Minnesota State Department of Health has revealed epidemiologic data of fundamental importance. This information is based upon 268 bacteriologic proved cases of human brucellosis. The data emphasize that brucellosis is primarily a disease of males, particularly of

farmers and packing plant workers. Children under 12 years of age are relatively resistant to this disease. This also applies to young cattle, young goats and young hogs. In the acquisition of human brucellosis in Minnesota, contact with infected material is much more important than acquiring the disease through drinking raw milk. The data indicate that for every case acquired through the ingestion of milk, there are four cases caused by direct contact. With the exception of fresh cottage cheese and fresh goat's cheese, it is doubtful that milk products play a very significant role in spreading human brucellosis. In a period of 12 years in the University Clinics, not a single instance has been seen where the disease was contracted through the ingestion of cheese, butter, or ice cream.

The nature of the acute illness in man may be likened to influenza, except that there are no respiratory symptoms. The outstanding manifestations of the disease are weakness, easy fatigability, generalized body aches and pains, headache, backache, nervousness, inability to sleep and emotional instability. The majority of cases do not endure beyond 3 months. Occasionally chronic disease may ensue with localization of the *Brucella* in certain tissues, including the bones, particularly the spine; the central nervous system, including the meninges; and on the valves of the heart. In this part of the country at least, brucellosis is rarely the cause of death.

During the past 12 years at the University of Minnesota Hospitals and Laboratories, efforts have been made to treat patients with antibrucella agents. Vaccines or filtrates of *Brucella* are not used in the treatment of brucellosis in our Clinics. Early experimental work indicated that *in vitro* the sulfonamides were active against the

Brucella. Disappointing results were obtained in patients with the use of all the sulfonamides as they appeared. Penicillin is without effect in brucellosis. Some hope was held out when streptomycin became available, but this drug when used alone is not too effective. A number of patients have been successfully treated with a combination of streptomycin and sulfadiazine. Unfortunately the streptomycin in some instances resulted in toxic reactions, and there was a relapse rate of about 20 to 25 per cent. In more recent months aureomycin, a new antibiotic, has been used successfully in the treatment of both acute and chronic cases of brucellosis. This drug is given by mouth, and, therefore, it is not necessary to hospitalize the patients. Treatment is continued for 10 days to 2 weeks. It would appear that both in Minneapolis and in Mexico from 80 to 90 per cent of the patients have been successfully treated. Reference is made only to bacteriologic proved cases. It appears that aureomycin is effective in human brucellosis caused by all three species of *Brucella*. More recently, chloromycetin, or chloramphenicol, has been made available for therapy. Indications are that this drug, which can be given by mouth, also is effective in the treatment of brucellosis.

Brucellosis as a human disease can only be eliminated by eradicating the infection at its source, that is, in domestic animals. Vaccination of young calves with *Brucella* is one effective means of controlling the disease in animals. Another procedure is to eliminate positive reactors from herds. The eradication of brucellosis in domestic animals will only be accomplished by a cooperative effort on the part of farmers, livestock producers, dairymen, public health agents, veterinarians, and physicians.

BUSINESS MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Minneapolis, Minnesota, June 23, 1949

President Petersen called the meeting to order at 3:00 p.m. in the auditorium of the Museum of Natural History. There were 175 present.

REPORT OF THE EXTENSION SECTION

The opening session of the program of the Extension Section of the 44th annual meeting of the American Dairy Science Association was called to order by Chairman Heebink of West Virginia on Tuesday, June 21, 1949 at 1:30 p.m. in Room 320 of the Coffman Memorial Union. Following his opening remarks and announcements, the following nominating committee was appointed: R. G. Connally of Virginia, R. A. Cave of South Dakota, and W. T. Crandall of New York as

Chairman. This session was devoted to the presentation of five papers dealing with various phases of Dairy Herd Improvement Associations.

Vice-chairman Reaves opened the Wednesday morning, June 22 session at 9:15 a.m. Those papers on teaching methods were presented and discussed. The section then retired to the room where several state exhibits on methods were presented under the chairmanship of Boynton of New Hampshire. State exhibits were explained by representatives of that state.

A joint session of the Production and Extension sections, L. A. Moore, Chairman, was held Wednesday afternoon. A panel discussion on the job of Herd Improvement was held with

Taylor of Pennsylvania State College as leader. Following the panel discussion Co-chairman Heebink conducted a session devoted to committee reports.

The Breeds Relations Committee and Herd Health Committee reports are incorporated in the report of the Production Section.

The Dairy Breeding Committee, E. J. Perry of New Jersey, Chairman, made the following recommendations:

1. That the A.D.S.A. place more emphasis on the Breed Herd Test and D.H.I.A. testing programs to promote testing of entire herds. Further, that H.I.R. records from all breed associations and all D.H.I.A. records be reported promptly to the U. S. Bureau of Dairy Industry, so that less confusing and a more complete proof will be published on all bulls.

2. That all sire proofs: preliminary, complete, H.I.R., private, etc., be properly designated as to what they are at the source of the proof.

3. Recommend that a licensed Veterinarian who is not a full time employee of an artificial breeding organization may collect blood samples for blood typing of sires. This is to be done in the presence of the manager of the breeding organization and both sign as to having properly identified each bull. This recommendation is designed to make unnecessary the presence of a breed representative at such times.

4. That the problem of identifying registered cows after service because the registration papers were not available is not properly the responsibility of the technician nor the artificial breeding organization.

5. Once a registered cow has been identified by her registration papers and ear tag at time of first service, it is recommended that the ear tag alone shall be sufficient identification on additional services.

6. Recommend that the secretary of Purebred Dairy Cattle Association consult with State Extension dairymen whenever interpretations of P.D.C.A. rules arise in that Extension dairyman's state.

7. Recommend that the American Dairy Science Association establish a committee to formulate practical recommendations to be used as a guide in setting up a sound artificial breeding program based on genetics.

The Type Classification Committee with Tyler of West Virginia acting as Chairman made the following recommendations:

1. The literature on studies of type be reviewed.

2. An evaluation of type programs and studies be considered as a topic for a panel discussion before a joint session of Extension and Production

Sections at the 1950 annual meeting of the American Dairy Science Association.

3. All animals in college and experiment station herds be classified at least once a year. If feasible all animals over 6 months of age should be included. The purpose of this recommendation is to make available some needed data for research purposes.

4. Wherever individual classifications ratings are published the age of the animal at the time of the rating be given.

5. Cooperation of persons in charge of college and experiment station herds be given this committee for a proposed survey on the utilitarian aspects of certain type characters assumed to be defects.

6. Cooperation be extended to the committee of the Purebred Dairy Cattle Association that is charged with the consideration of type problems.

7. The name of this committee be changed to the Type Committee.

Thursday, A.M., June 23.

Chairman Heebink introduced the speakers presenting papers on 4-H club work. Following discussion of these papers, Chairman Heebink opened the business session of the Extension Section.

Six committee reports were read, amended and approved. The nominating committee brought in a slate of two candidates for Secretary of the Extension Section for 1949-50. Ramer D. Leighton of Minnesota was elected Secretary.

Chairman Heebink called the final session to order at 1:30 p.m. at which time two papers on artificial breeding results and sire selection were presented. The section was then adjourned to the business meeting of the Association.

Respectfully submitted—G. HEEBINK, *Chairman*; C. W. REEVES, *Vice-Chairman*; RAYMOND ALBRECHTSEN, *Secretary*.

Upon motion duly seconded, the report was accepted.

REPORT OF THE PRODUCTION SECTION

The Production Section held eight sessions at which 70 papers were presented. Eight sessions were held with concurrent sessions held to provide for the large number of papers, as customary for the past 2 years. Two business meetings were held, and presided over by Chairman L. A. Moore.

In addition to the eight sessions at which papers were read, a session was held with the Extension Section at which a panel discussion on the job of Herd Improvement, lead by Joe Taylor was held. Other participants were E. E. Heizer on "Allowing for the Effect of Environment in Production", J. L. Lush on "Estimating

the Breeding Value of Young Bulls", G. A. Bowling on "Should a Bull be Linebred or Outbred?", V. A. Rice on "What about Indexes in the Selection of Bulls?", Milton Fohrman on "Results from Crossbreeding", and Frank Astroth on "Reasonable Production Increase to be Expected from Culling."

The reports for the following committees were presented and accepted by the Production and Extension Sections in joint session.

Breeds Relations presented by H. A. Herman, Chairman, D. L. Fourn, W. W. Yapp, Floyd J. Arnold, E. C. Scheidenhelm and Lynn Copeland.

Action recommended by the committee follows:

Rule 3, page 7—Change the following sentence "A supervisor is limited to 36 milkings per day" to read "The number of milkings supervised daily shall not exceed 48".

Rule 12, page 18—Change to read as follows: "The number of milkings supervised per day A—When the herd is enrolled in a Dairy Herd Improvement Association and the tests are used for both H.I.R. and D.H.I.A., the number of milkings shall not exceed 60 per day, B—In central testing laboratories where additional technicians are working the number of cows supervised by each supervisor shall be limited to 90 milkings, and C—When the tests are used solely for the H.I.R. report the number of milkings supervised shall not exceed 90 per day".

Rule 15, page 18—Change the following sentence "The retest shall include the entire herd, or, in case of herds requiring more than one day's supervision that portion of the herd tested with the qualifying animals during the regular test period" to read "The retest shall include the entire herd, or, in case of herds requiring more than one day's supervision, only those cows meeting retest requirements need to be included in the retest".

The committee recommends that the above revisions be made effective July 1, 1949.

Dairy Cattle Health presented by C. G. Bradt, W. E. Petersen, Chairman, W. D. Pounden, R. E. Horwood, Joe Nageotte and W. R. Walker.

Dairy Cattle Breeding—reported by Extension Section (Type Classification)

Pasture Investigation Technique presented by R. H. Lush, Chairman, J. B. Shepherd and W. B. Nevens.

Special Report of Dairy Cattle Judging Coaches—Fordyce Ely, Chairman.

Dairy Cattle Judging—S. M. Salisbury, Chairman, R. E. Johnson and D. L. Fourn, read by section Secretary.

It was voted that the two above committees and others that they may have represented be

dismissed, and that in their place a new committee to be called the National Intercollegiate Dairy Cattle Judging Contest Committee be appointed to carry on with these activities.

Resolutions by D. M. Seath, chairman; R. E. Hodgson and K. L. Turk.

Other business—Suggestions for improving the meetings included:

1. The use of mimeographed summaries and graphs available in the abstracts.

2. Less use of slides when mimeographed material is feasible.

3. Placing available mimeographed material at a central location for the convenience of those not attending the particular section in which the paper is given.

Nominating Committee. The report of this committee by G. W. Salisbury resulted in the subsequent election to the office of Secretary of N. N. Allen, University of Wisconsin.

There being no further business, the meeting adjourned.

Respectfully submitted—L. A. MOORE, *Chairman*; G. M. CAIRNS, *Vice-Chairman*; L. O. GILMORE, *Secretary*.

Upon motion duly seconded, the report was accepted.

REPORT OF THE MANUFACTURING SECTION

The program for the manufacturing section was carried out as scheduled and published in the May issue of the JOURNAL OF DAIRY SCIENCE. A total of 38 submitted papers and a symposium on milk proteins consisting of four invitational papers were presented. Three papers (M10, M21 and M22) were not given. The symposium on milk proteins was under the leadership of A. M. Swanson.

The business meetings of the section were held Tuesday, June 21 at 4:30 p.m. and Thursday, June 23 at 11:00 to 12:00 a.m., with Chairman E. M. Barker presiding. Reports from the Standing Committees on Butter, Dairy By-Products, Milk and Cream with six subcommittees on Products Judging, on Standardizing Methods for Conducting all Phases of Babcock Testing, and on Standardizing all Tests of Dairy Alkalis and Methods of Reporting Results, were read and accepted.

The following motions were passed:

1. That the scoring method for sediment in bottled milk and cream proposed by the committee be accepted as official by the section and presented to the general session for adoption.

2. That the work of the following committees be continued:

- (a) Committee on Standardization of the

Acidity Test of all Dairy Products.

(b) Committee on Standardization of all phases of Babcock Testing.

(c) Committee on Standardizing Dairy Alkali Tests.

(d) Committee on Dairy By-Products.

3. That a committee be appointed to study methodology, classification and nomenclature of milk proteins.

The section elected the following officers to serve for the coming year: E. L. Jack, Secretary; J. H. Hetrick, Vice-chairman; and D. V. Josephson, Chairman.

Respectfully submitted—E. M. BARKER, *Chairman*; D. V. JOSEPHSON, *Vice-Chairman*; J. H. HETRICK, *Secretary*

Upon motion duly seconded, the report was accepted.

EDITOR'S REPORT

The twelve issues of Volume XXXI of the JOURNAL OF DAIRY SCIENCE printed during 1948 consisted of 924 pages of original articles, 8 pages of Association announcements, 20 pages of program for the annual meetings, 32 pages of proceedings of the annual meetings, 73 pages of abstracts of papers presented at the annual meetings, 46 pages of indices, 37 pages of membership list, 160 pages of abstracts and 3 pages of miscellaneous. This makes a total of 1,303 pages, exclusive of the advertising sections and blank pages.

The material printed included 102 manuscripts (54 in the production field and 48 in the products field) and 4 reviews (1 in the production field and 3 in the products field), 137 abstracts of papers presented at the annual meetings and 436 abstracts of literature appearing in the Abstract Section. Of the 130 papers submitted for publication during the year, 14 were rejected and 55 were on hand at the end of the year in various stages of processing for publication.

The assistance of all those who have aided in the review of papers is acknowledged with gratitude. Without the continued cooperation of the reviewers present standards could not be maintained. The cooperative manner in which authors have helped to maintain publication standards and to condense their material as much as possible also is appreciated.

In line with the recommendation of the Journal Management Committee, the Abstract Section has been expanded as of January 1, 1949, by the addition of a considerable group of new abstractors and by the appointment of section editors for the abstracts. As of April 1, 1949, 57 abstractors had agreed to abstract 99 journals and additions to this group will be made as the

opportunity arises. In the first five issues this year, 417 abstracts have been published. This expansion in coverage has been possible only because of the excellent cooperation which many members have given this project. The two-column format of the Abstract Section also was adopted with the January issue. The slightly narrowed margins and slightly smaller type have resulted in a considerable saving in paper and press costs without sacrificing readability of the of the abstracts. Any members who have access to significant journals which now are not being covered in the Abstract Section are invited to volunteer for abstracting such journals. The assistance of abstractors familiar with one or more of the foreign languages, especially Italian, French, German and Finnish, is especially needed at present.

Respectfully submitted—F. E. NELSON, *Editor*

Upon motion duly seconded, the report was accepted.

SECRETARY-TREASURER'S REPORT

The membership and circulation of the JOURNAL for the year 1948 showed a further rise in numbers totaling 3881 by the end of the year. This total is made up of 1747 members, 780 student affiliates and 1357 subscribers.

The following is a summary of our gains and losses in members for 1948:

Membership December 31, 1947	1663
Gains: New Members 1948	177
Former student affiliates	18
Total gain	195
Losses: Members resigned	12
Members delinquent	92
Members deceased	7
Total loss	111
Net Membership gain	84
Membership Total, December 31, 1948	1747

In order to supplement the membership drive which was conducted last year and to increase the membership of the association for 1949, your secretary will be glad to write a personal letter from this office to any prospective members whose names may be sent to him. It is hoped that this may result in a larger membership in 1949 and aid in the membership efforts of our present members.

Another phase of membership which needs further attention by all members and especially by those who are at universities in the country, is the encouragement of student affiliates to assume full membership in the association upon graduation. Here is a source of members which has never been fully realized and never fully drawn upon by the association.

Plans are under way to contact all dairy school graduates with literature regarding the associa-

tion and the advantages of membership therein, but if such a program is to be successful, all persons who are in contact with such graduates must make every effort to get the story of the American Dairy Science Association across. Close cooperation between dairy department heads and the secretary should assure success.

The biggest worry of the Association is the ever increasing cost of JOURNAL publication. It might be well to mention that unless some method is devised for defraying or lowering the cost of publication, the association can expect to operate in the red for this year the same as it did for the year just past. Some of the ways in which this cost can be overcome is through increased membership, an increase in the number of subscribers, and an increase in advertising carried.

Advertising which appeared in the JOURNAL during 1948 occupied 199 pages and was worth \$7,840.64 to the association as compared with 198 pages and \$7,225.92 for 1947. Your secretary feels that the low advertising rates which we are charging should induce more companies to place advertising in the JOURNAL and that we should engage in an active program of solicitation for the year 1950. Any ground work which the members can give along this line will be greatly appreciated and will do much to assist with the financial problems of the Association.

It might be of interest to the members to know that we have 506 foreign subscribers to the JOURNAL. The big three in the foreign field are Australia, England and Holland with 56, 73 and 52 respectively. Altogether our JOURNAL is being sent to 45 foreign countries, so you can see that interest in dairy science is world wide.

Sale of back copies during the past year totaled 570. This was 220 below 1947, and followed an anticipated trend downward inasmuch as most of the members and subscribers who missed out on issues during the war have purchased the back copies they desired. There are some issues which are out of print at present which undoubtedly should be reprinted at the earliest possible moment of suitable cost. To date the association has made no move in this direction feeling that it would be wiser to wait until printing costs come down a bit.

The student branches of the American Dairy Science Association are showing continued and increasing activity and are fast becoming a factor in the world of dairy science. Since our meeting last year, the Executive Committee has granted certificates to the following schools: Rutgers University, New Jersey, a new certificate; University of Massachusetts, Ohio State University and the University of Georgia, renewal certi-

cates. While we are on the subject of student branches, an apology is in order for Clemson College. The name of Clemson was omitted last year from the list of those schools which had student branches through an oversight. Clemson, under the able leadership of J. P. LaMaster and B. E. Goodale, is definitely to be numbered amongst the living.

The big four in student activity in the American Dairy Science Association are Ohio with 148 students, Oklahoma with 68, Iowa with 66 and Wisconsin with 51. Ohio leads the field with more than twice as many members as its nearest rival. Apparently W. L. Slatter and S. M. Salisbury must have the secret of successful chapters well in hand. Last year's total student affiliate membership was 780. So far this year the total has increased to 846, with new memberships coming in frequently.

As per predictions made by the late R. B. Stoltz at our Athens, Georgia meeting, your Association operated at a \$3,083.37 loss during the past year. This loss was entirely unavoidable due to the already high and ever increasing costs of printing the JOURNAL. The executive board has set up a budget of \$39,500.00 for 1950 which is considerably higher than the 1949 budget, and reflects this increased cost of operation. Our net worth as of December 31, 1948, was \$36,036.84. A complete report of the Certified Public Accountant was sent to each member of the Executive Board in March. One bright spot in the financial picture for the year was the maturing of three \$1,000 bonds which your secretary reinvested by purchasing four \$1,000 Series F bonds which are now in the Association's Safe Deposit Box. The interest from the three bonds totaled \$790.00 which enabled us to purchase an additional bond and place the extra \$40.00 in our bank account.

I wish to take this opportunity to express my sincere and grateful thanks to all those members of the Association, who through their willingness to help and the encouragement which they have offered, have made the job of acting Secretary-Treasurer both instructive and enjoyable.

Respectfully submitted—P. R. ELLSWORTH,
Acting Secretary-Treasurer.

Upon motion duly seconded, the report was approved.

AUDITING COMMITTEE REPORT

The president then requested the report of the Auditing Committee which was read by H. S. Willard.

May 11, 1949

To the Directors and Members of the

American Dairy Science Association
Gentlemen:

On May 6, 1949, Mr. Walter C. Burnham, a certified public accountant, met with the Auditing Committee of the American Dairy Science Association. At that time, Mr. Burnham's report of his audit of the American Dairy Science Association business for 1948 was considered.

Mr. Burnham has made a thorough examination of the records. He has checked the bank statements and examined all the U. S. Government Bonds. Mr. Burnham had check-tested the inventory of Journals and Twenty-Year Index to assure accuracy of the physical inventory.

The Auditing Committee is satisfied that the financial statement for the year 1948 is correct. We recommend that it be accepted by the Board of Directors and the members of the American Dairy Science Association.

Respectfully submitted—W. J. BRAKEL, *Chairman*; R. N. KENNEDY; H. S. WILLARD.

Upon motion duly seconded, the report of the Auditing Committee was accepted and ordered filed.

REPORT OF THE JOURNAL MANAGEMENT COMMITTEE

During the past year as authorized by the Journal Management Committee and the Executive Board of the American Dairy Science Association, the following action has been taken.

1. The abstract section of the editorial work has been reorganized and section editors in the various subject matter fields appointed.

2. The abstract section of the Journal is now printed in two-column pages and in slightly smaller type thus saving considerable space.

3. Two exchanges with foreign journals have been arranged and others are being contemplated.

In furtherance of a program of continued progress the Journal Management Committee recommends:

1. In continuation of the policy of rotating the associate editors the Journal Management Committee recommends that Associate Editors H. A. Ruehe and E. P. Reineke be retired and W. V. Price and H. A. Herman be appointed as replacements.

2. In view of the possible desirability of expanding the exchange arrangement with foreign journals for abstracting purposes the Journal Management Committee recommends that the Executive Board authorize the provision of sufficient exchange copies as needed for this purpose.

3. The Journal Management Committee recommends that the Proceedings of the Annual Meeting and the Abstracts of Papers presented

at the Annual Meeting be printed in two column pages in the same form as is now used for the abstract section.

4. In consideration of the high printing costs and little prospect of relief from these high costs in sight, the Journal Management Committee recommends that the Executive Board consider retrenchment or action on certain proposals to increase the revenue of the Association.

The Journal Management Committee wishes to express the commendation of the Association membership to the Editor and Editorial Staff in acknowledgment of the excellence of their work.

Respectfully submitted—T. S. SUTTON; P. R. ELLIKER; G. H. WISE.

Upon motion, duly seconded, the report was approved.

REPORT OF NATIONAL RESEARCH COUNCIL

The activities of the Division of Biology and Agriculture of the National Research Council are varied and numerous. Several are of particular interest to the American Dairy Science Association. At present this Division has committees studying the problems involved in the public health aspects of Brucellosis in animal nutrition and in milk production, distribution and quality.

Since the organization of the American Institute of Biological Sciences, about two years ago, under the sponsorship of the National Research Council, there has been some merging of the programs followed by the Institute and the Division of Biology and Agriculture. This is inevitable since it was designed in the organization of the Institute that all member Societies of the Division would become members of the Institute.

As of January 1, 1949, the Institute had fifteen members and two societies as affiliates. Already during this year several other societies have become members of the Institute, thus adding to its strength and usefulness.

The American Dairy Science Association as yet has not joined the Institute. An invitation was issued by the Institute to our Association to have a representative at its board meeting on May 4, 1949, so President Petersen requested your reporter to attend. Much of the report made at this meeting was again reported and discussed at the meeting of the Division of Biology and Agriculture which was held the next day.

Items that should interest our membership were: (a) A report of the Committee on Advisory Services to Armed Forces, (b) A report of the Committee on Handbook of Biological Data, and (c) A report of the Publications Committee.

The Committee on Advisory Services to the Armed Forces has been asked by General Hershey, Director of Selective Service, to serve as his advisory panel in the field of the biological sciences. This is the first time that biology, as such, has been officially recognized in connection with manpower problems in the U. S. A plan has been proposed for organized cooperation between the Armed Services and biology in placing scientific personnel where it can serve most usefully. Under it, all worthy young scholars will be deferred. The tests for deferment are (a) interest in a scientific field and (b) an intelligence level at least equal to that required for officer training. If this latter test produced too many deferments, then the intelligence standards would be raised. These intelligence standards would be applied and the weeding out done within each college or university.

A proposal was also made to put all scientists in a common pool—probably with a common science uniform, so that they could be transferred from one service to another with no difficulty and placed where they could do the most good.

The plan as finally worked out has been accepted by the Selective Service Administration and forwarded to the President of the United States for his approval and action, probably through a Presidential Directive.

The idea for a Handbook of Biology really started when the Air Force asked for a Handbook on Medical Science. Due to the efforts of the American Institute of Biological Sciences, the scope of the Handbook was enlarged and the Institute given the responsibility of its compilation. The Air Force has underwritten the start of the book through an appropriation of \$15,000 but the ultimate cost is expected to be about a quarter of a million dollars. The Handbook will contain authentic, accepted and frequently used constants, data, normal values, tolerances, and standards applying to the quantitative aspects of biology in its broad and basic sense, including many fields of applied biology. The committee in charge of this compilation solicits the cooperation of all interested societies and their members in gathering the material that each would most like to see in a handbook.

The Publications Committee has been studying the economies that might occur through cooperative publication of magazines of member societies. In canvassing the membership of the Division of Biology and Agriculture it was found that twenty of the Societies had a lively interest in this venture, eighteen were somewhat interested and twelve were not interested. Further efforts will be made to serve those interested Societies by getting cost of publishing their magazines uni-

formly as to size, kind of paper, by standard printing or by the off-set method, etc.

During the past year Dr. R. E. Cleland, Department of Botany, Indiana University, replaced Dr. J. S. Nicholas as Chairman of the Division of Biology and Agriculture. Dr. Cleland also was Chairman of the Board of Governors of the American Institute of Biological Sciences during the past year, but was replaced at the last meeting by Dr. E. G. Butler, Department of Biology, Princeton University.

Respectfully submitted—C. Y. CANNON

Upon motion duly seconded, the report was approved.

NECROLOGY COMMITTEE REPORT

Robert Bear Stoltz, Professor and Chairman of the Department of Dairy Technology, Ohio State University, Columbus, passed away on October 2, 1948. He was born on March 6, 1890, at Bradford, Ohio. He graduated from Ohio State University in 1912 and joined the staff of that University soon after graduation. He taught Dairy Husbandry and was promoted to full professor in 1923. In 1929 he was made Chairman of a newly formed Department of Dairy Technology, a position he held until his death. Professor Stoltz took an active part in the organization of several commercial dairy associations. The Ohio Swiss Cheese Association and the Columbus Milk Distributors Association are but two highly successful groups that he organized and served for many years. He also served as secretary of the National Cheese Association for several years and was an honorary member of the Board of Trustees of the Ohio Dairy Products Association. His outstanding service to the American Dairy Science Association is well known to all members. Aside from serving as Secretary of this Association for over 12 years he also was our president in 1934. His tireless efforts in promoting the affairs of the Association have made this Association a large, aggressive and financially sound organization. It is fortunate that this association recognized his many contributions before his untimely death by presenting him with the Association Award in 1947. Professor Stoltz was very active in Masonry work and attained the 33rd degree in that organization. Before his death he was elected to the post of Deputy General Grand Master of the General Grand Council, R. & S.M. of the United States. He is survived by his wife, Mrs. Marie Cassel Stoltz, one son and three daughters. His untimely death is a great loss to this Association but the fruits of his labors will long be felt in the progress of the American Dairy Science Association.

Samuel Irvin Bechdel, Professor of Dairy Husbandry, the Pennsylvania State College, State

College, was born at Howard, Pennsylvania, July 9, 1886. He received his B.S. degree from the Pennsylvania State College in 1911, an M.S. degree from the same institution in 1916 and a Ph.D degree from the University of Minnesota in 1925. During his 32 years of service to the Pennsylvania State College Dr. Bechdel distinguished himself as a teacher and scientist. His most outstanding contributions in the field of Dairy Research were his studies on vitamin requirements of dairy cattle and silage and pasture investigations. His experimental work with vitamin B synthesis in ruminants attracted world wide attention. Dr. Bechdel was very active in church work, serving as Deacon, Elder and as teacher of the Men's Bible Class of the Faith Reformed Church for some 20 years. He retired in January, 1946, due to ill health and passed away on September 13, 1948. He is survived by his wife and four children.

Christian Larsen was born in Odense, Denmark on August 4, 1874. After coming to this country he received a B. S. degree in Agriculture at Iowa State College in 1902 and an M.S. degree from that institution in 1904. He served on the instructional staffs at Massachusetts State College, Iowa State College and Utah State College and in 1907 went to South Dakota State College as Professor of Dairy Husbandry and Director of Extension. In 1921 he became Director of Dairy Marketing for the Illinois Agricultural Association. He was appointed Dean of Agriculture at the South Dakota College in 1923, a position he held until his retirement in 1940. Dean Larsen was one of the pioneers in the field of dairy science and made many early contributions in both dairy manufacturing and production. Production heredity, physiology of milk secretion, effects of water and alkali on nutrition and the chemistry of butter were but a few of his major interests. He was the author of several early books on dairying subjects among which were *Principles and Practices of Buttermaking*, published in 1905, and *Dairy Technology*, published in 1914. He held memberships in many Agricultural and Scientific societies, as well as fraternal and civic groups. He was a member of the American Dairy Science Association from 1920 until his death and was honored with a life membership in this Association in 1943. Dean Larsen passed away on August 23, 1948.

Thomas E. Elder was born in Virginia in October 1882 and died of a heart attack at Cedar Grove, N. J., September 8, 1948. He was graduated from Cornell University in 1911 where he specialized in Animal Husbandry under the late Professor H. H. Wing. Shortly after completing his college course, he joined the staff of the Mount Hermon School for Boys at Mount Her-

mon, Mass. and became Dean of the school in 1926. He served as a director of the Holstein-Friesian Association of America from 1921 to 1941, a period of 20 years. He was a member of the True Type Committee of the Holstein-Friesian Association, the committee that developed the true type models and paintings for the Holstein breed, and served as an official inspector from the beginning of the type classification program in 1929 until the day of his death. He had gone from his home in Alton, N. H. to inspect and classify the Overbrook Dairy herd at Cedar Grove, N. J. and there his death occurred. His wife, Grace Holton Elder, and two sons survive him.

Thomas B. Buchanan, manager of Borden's Hamilton Milk Co., Columbus, O. for over 28 years, died September 18, 1948. A native of Cincinnati, Mr. Buchanan spent his boyhood in that city, but moved to begin his career in the dairy industry in Columbus, O. as a route salesman for the Pure Milk Co. Mr. Buchanan later became a member of the Moores and Ross organization and was appointed manager of the Hamilton Milk Co. which they acquired in 1920. After the Borden Company acquired this company in 1929, Mr. Buchanan remained as manager of the expanded Borden's Hamilton Milk Co. and held this position until his death. Mr. Buchanan joined the American Dairy Science Association in 1932. At the time of his death he was treasurer of the Columbus Milk Distributors Association, treasurer of the Ohio Dairy Products Association, and a member of the Columbus Lions Club. He is survived by his wife, three daughters and two grandchildren.

It was reported to the Association that Mr. Charles Staff of Pleasant Ridge, Mich. passed away on July 10, 1948. Mr. Staff was formerly associated with the Larro Research Farms at Detroit, Mich. and at the time of his death was with General Mills at Pleasant Ridge, Mich. He has been a member of the Association since 1942. The committee was unable to obtain further information regarding Mr. Staff.

Respectfully submitted—J. P. LAMASTER; D. V. JOSEPHSON; D. M. SEATH, *Chairman*.

Upon motion duly seconded, the report was accepted.

RESOLUTIONS COMMITTEE REPORT

WHEREAS: The University of Minnesota through its administrative staffs and faculty has made available to the American Dairy Science Association in this its 44th Annual Meeting all needed physical facilities for the meeting, and

WHEREAS: Every possible personal courtesy has been given to members of the Association for their enjoyment and entertainment,

Therefore, be it **RESOLVED**: That the American Dairy Science Association take this opportunity officially to extend its thanks and appreciation and hereby request the President of this Association to convey by letter this appreciation to President J. L. Morrill and to Dean Clyde H. Bailey, and Professor J. B. Fitch.

WHEREAS: Many commercial and civic organizations have contributed greatly to the success and enjoyment of this 44th annual meeting,

Therefore, be it **RESOLVED**: That the American Dairy Science Association express to these organizations its sincere appreciation.

WHEREAS: The Borden Company has again offered its awards for outstanding research in dairy manufacturing and production,

Therefore, be it **RESOLVED**: That the American Dairy Science Association express to the Borden Company its sincere appreciation of this evidence of its continued interest in dairy research.

WHEREAS: The American Feed Manufacturers Association has seen fit to offer an award for outstanding research in the field of dairy cattle nutrition,

Therefore, be it **RESOLVED**: That the American Dairy Science Association express to the American Feed Manufacturers Association its sincere appreciation for their interest in and encouragement of research in dairy cattle nutrition.

WHEREAS: The Purebred Dairy Cattle Association has continued in its cooperation with the American Dairy Science Association in establishing uniform rules for the testing of dairy cattle,

for the regulation of artificial breeding and other matters promoting uniformity and,

WHEREAS: The Purebred Dairy Cattle Association has established a Dairy Cattle Breeding Research Council for the purpose of encouraging and supporting research in this field, in cooperation with the various experiment stations,

Therefore, be it **RESOLVED**: That the American Dairy Science Association commend the Purebred Dairy Cattle Association for its efforts.

WHEREAS: The officers of the various sections of the Association are changed annually and are often unfamiliar with organizational procedure, considerable confusion and misunderstanding exists.

Therefore, be it **RESOLVED**: That the Executive Board instruct the Secretary of the Association to prepare a report outlining the setup of the Association and the procedures to be followed in handling committee reports, recommendations and resolutions by the various sections in order that the business of the association at the annual meeting can be handled in an orderly manner.

Respectfully submitted—A. A. SPIELMAN, Chairman; F. J. ARNOLD; F. C. FOUNTAINE; H. B. HENDERSON; A. J. MORRIS.

REGISTRATION COMMITTEE REPORT

S. T. Coulter, University of Minnesota, made the following report for the Registration Committee. Upon motion duly seconded, it was accepted.

C. Y. Cannon moved and A. W. Rudnick seconded that all actions of the Executive Board during the past year be approved.

Alabama	2	Maryland	32	Oklahoma	10
Arizona	2	Maine	3	Oregon	3
Arkansas	5	Massachusetts	15	Pennsylvania	37
California	16	Michigan	32	South Carolina	5
Colorado	2	Minnesota	198	South Dakota	14
Connecticut	6	Mississippi	3	Tennessee	8
Delaware	2	Missouri	26	Texas	6
Washington, D. C.	23	Montana	6	Utah	6
Florida	5	Nebraska	16	Vermont	10
Georgia	1	New Hampshire	4	Virginia	10
Illinois	93	New Jersey	14	Washington	12
Indiana	29	New Mexico	3	West Virginia	8
Iowa	68	New York	70	Wisconsin	111
Kansas	14	North Carolina	19	Wyoming	4
Kentucky	44	North Dakota	6		
Louisiana	5	Ohio	84		

U. S. Territories

Hawaii	3
Puerto Rico	4

Foreign Countries

Australia	6	India	4
Belgium	1	New Zealand	1
Canada	2	Scotland	1
		Turkey	1

Men Present	780
Women Present	262
Children Present	73

Total registration 1115

MEETING OF THE EXECUTIVE BOARD AMERICAN DAIRY SCIENCE ASSOCIATION

P. R. ELLSWORTH, *Secretary-Treasurer*

The Executive Board transacted the following business:

Approved the minutes of the past annual meeting.

Approved the Editor's report.

Approved the Secretary's report.

Approved the Journal Management Committee report.

Approved the Auditing Committee report.

Approved the Budget for 1950, amounting to \$39,500.

Received the report of the representative of the National Research Council.

Re-employed the editor for the ensuing year.

Employed the present acting Secretary-Treasurer as Secretary-Treasurer for the ensuing year.

Voted unanimously to make H. B. Ellenberger an honorary member of the Association.

Elected J. K. Loosli as a member of the Journal Management Committee to serve for the three ensuing years.

Accepted Cornell University's invitation to hold the 45th Annual Meeting of the Association there in 1950.

Voted to make O. C. Cunningham a life member of the Association.

Voted to recommend that the Association no longer furnish free reprints of articles to authors.

Voted to recommend to the Association that:

Domestic subscription rates be raised from \$6.00 to \$10.00 per year.

Foreign subscription rates be raised from \$6.50 to \$10.50 per year.

Membership dues be raised from \$5.00 to \$6.00 per year.

Student Affiliates dues remain at \$3.00 per year.

The Associate Subscriber classification be discontinued and that all present associate subscribers be invited to become members or subscribers as the appropriate case may be, with no \$5.00 affiliation fee being charged those who become members.

Voted to have printed abstracts of all papers presented at the annual meeting available at the registration table. Cost of these reprints to be included in the registration fee. The journal will print all abstracts in the June issue.

Renewed student branch certificates for the University of Massachusetts, University of Georgia and Ohio State University and issued a new certificate to Rutgers University, New Jersey.

Went on record as being in favor of the establishment of some sort of an award for Extension personnel should a suitable donor be found.

Empowered Journal Management Committee to make final decisions, if needed, relative to new or questionable advertising.

Approved the Resolutions Committee report as corrected.

Recommended to the Association that the Secretary be authorized to accept applications for membership in the American Dairy Science Association from citizens of foreign countries who have received advanced technical training in dairy science in the United States or Canada. This training would normally be expected to represent the equivalent of a Master's degree or Doctor's degree in our colleges, or work of at least one year's duration towards such degrees.

Recommended to the Association that student affiliate memberships of foreign citizens in United States or Canadian colleges may be converted to full memberships by the usual method of conversion now used for United States student affiliates.

Recommended that a new class of foreign members be established as follows: Upon the nomination of two or more members and approval by the Executive Board, distinguished foreign dairy scientists may be elected to membership with all the privileges of domestic members except that they have no voting privileges. The number of members elected normally would be restricted so that such election would be considered a distinct honor.

Recommended that the Association print the lectures of Dr. W. W. Spink on "Brucellosis in Man" and by Dr. Ancel Keys on "Cholesterol and the Problem of Aging" in the JOURNAL OF DAIRY SCIENCE as part of the proceedings of the annual meeting.

The Nominating Committee consisting of Warren Gifford, P. H. Tracy, E. G. Hood, Otto Hill and C. G. Bradt nominated the following candidates in April: Vice-president, R. B. Becker and S. J. Brownell; directors, C. W. Turner, J. P. LaMaster, F. J. Arnold and E. J. Perry.

Results of the election were announced on June 1 as follows: Vice-president, R. B. Becker of Florida; directors, C. W. Turner of Missouri, and F. J. Arnold of Iowa.

Upon motion duly seconded the report was approved.

THE AMERICAN DAIRY SCIENCE ASSOCIATION AWARDS

Minneapolis, Minnesota, June 23, 1949

W. E. Petersen, of the University of Minnesota, acted as toastmaster at the annual awards banquet on June 23 at Coffman Memorial Union, Minneapolis, Minnesota. He installed the officers-elect as follows: G. M. Trout, of Michigan, was installed as President; R. B. Becker, of Florida, as Vice-President; C. W. Turner, of Missouri, and F. J. Arnold, of Iowa, as Directors.

Mr. Trout, you are about to take over the responsibilities of President of the American Dairy Science Association. As President it will be your duty to be chairman of the Executive Board and submit to the Board for approval the nominations of members to fill vacancies that may occur among the elected officers of the Association. As President you shall appoint, without the approval of the Executive Board, the standing non-elective committees of the Association. With these obligations, privileges and responsibilities I now charge you with the honor of being President of the American Dairy Science Association with all the privileges, responsibilities and obligations pertaining thereto.

Mr. Becker, you are about to take over the responsibilities of Vice-President of the American Dairy Science Association. As Vice-President, it will be your duty to preside over the Executive Board in the absence of the President and assume other duties of the Executive Board. At the expiration of President Trout's term, you will automatically become President of this Association. I now charge you with these duties.

Mr. Turner and Mr. Arnold, you were elected to the Executive Board of the American Dairy Science Association. It is the duty of the Board members to pass on all applications for the establishment of divisions, sections, and student branches of the Association. You will have full control of the budget and general business of the Association and have title to all property and funds of the Association. You will be members of the Board that has all the rights and power vested in the by-laws of the Association. With these privileges, responsibilities, and obligations you are now considered as members of the Executive Board of the American Dairy Science Association to serve a term of three years.

PRESENTATION OF ASSOCIATION AWARD

The toastmaster then introduced J. A. Nelson, chairman of the Association Honors Committee, who made the following citation:

Howard Bowman Ellenberger was born at Dallas, Iowa and educated in the public schools

of Iowa. He entered Iowa State College and was granted the Bachelor of Science degree by that institution in 1905. He earned the Master of Science degree from Cornell University in 1915 and continued with his advanced studies and investigations at Cornell to earn the degree of



HOWARD BOWMAN ELLENBERGER

Doctor of Philosophy in 1917. He married Priscilla Flatt, who has been a very conscientious and ardent co-worker in his professional life.

Before graduation he was undergraduate work, Howard Ellenberger gained practical experience by working as herdsman in Illinois and in Minnesota before graduating from Iowa State College. After graduation, he gained further practical experience as foreman and superintendent of farms in Illinois, Missouri and Iowa. His quest for further knowledge and his ambition led him to Cornell University in 1914 as instructor and graduate student. During the time he was instructing and doing graduate work at Cornell, he served as specialist in dairy manufacturing during the summer vacations for the Vermont Department of Agriculture. He performed his duties so well that he was offered and accepted the position as Associate Professor of Animal and Dairy Husbandry at the University of Vermont after

completing the work for the doctorate in 1917. His efficiency, sincerity and foresight brought about rapid advancement and in 1918 he was made head of the Department of Animal and Dairy Husbandry, a position which he held until his retirement in 1948.

Doctor Ellenberger has had a wide field of interest. He was author and co-author of a number of dairy bulletins and scientific papers on dairy production, dairy cattle management, dairy feeds, breeding and dairy products manufacturing and regulatory work. His interest and influence in the dairy industry went far beyond the borders of his home state of Vermont. He visited other institutions to study their methods and to exchange ideas with other workers. He always was helpful in assisting others with their problems.

His prominence and his good judgement were recognized by others in the dairy industry. He was honored by being selected as chairman of the New England Governors' Dairy Advisory Board to organize and administer the Boston market milk area from 1931 to 1933. This duty he performed with distinction.

To achieve closer coordination of the Vermont dairy industry, he organized the Vermont Dairy Plant Operators and Managers Association of which he was executive secretary from 1921 to 1941. He is now honorary secretary of this association. Professor Ellenberger has always been a diligent and sincere worker in the American Dairy Science Association. He served as chairman of the Eastern Division in 1926, chairman of the Production Section in 1930 and was president of the National Association in 1931 and director in 1940-42. His interest in scientific research and advancement was not limited to the dairy field. He has been a member of the American Society of Animal Production in which he served as North Atlantic vice-president and as chairman of the North Atlantic Section.

For these outstanding achievements in the dairy field and in other related fields and for the long and honorable service in the advancement of the dairy industry, it is with great pleasure that the American Dairy Science Association selects Howard Bowman Ellenberger as "Dairyman of the Year". In token of our high esteem of his professional accomplishments, we present to him this distinguished service award.

PRESENTATION OF BORDEN AWARDS

I. A. Gould, member of the Borden Award Committee for manufacturing, was then introduced and made the following statements:

The man chosen for the 1949 American Dairy Science Association Borden Award in Dairy Manufacturing has for more than a quarter of a

century been contributing to our knowledge in this field of endeavor. Over this span of years he has conducted a wide variety of research, both fundamental and applied, which has revealed unusual creative ability and a broad knowledge in dairy technology and in the allied science fields. His contributions have been based on sound scientific techniques and procedures which have yielded results of much value to the dairy industry.



FRANCIS JANNEY DOAN

This man has been author or co-author of more than eighty publications, the major part of which pertain to original research. His research and publications have been principally in the fields of market milk and concentrated milk products. He was a pioneer in studying homogenization and has conducted research of this process, as applied to milk and cream, for more than 22 years. In these studies he demonstrated the effect of homogenization and of fat clumping on the heat and alcohol stability of milk proteins and obtained information on the fundamental factors responsible for the heat "curdling" or "feathering" of cream. Also, his research revealed that the lipase enzyme in milk was inactivated at relatively low temperatures, so that this enzyme was not a factor of spoilage in pasteurized homogenized milk. In later studies dealing with the stability of emul-

sion of fat in homogenized milk, he obtained results which served as the basis for establishing new standards for the commercial bottled product.

Another important phase of his research has dealt with soft curd milk. By utilizing an improved *in vitro* technique for determining the digestibility of milk and for judging its suitability for infant feeding, he demonstrated the treatments and processes which yielded milk with superior digestible characteristics. His work tended to cast considerable doubt on the reliability of "curd-tension" values in indicating the digestibility of milk. Also, in this series of studies, he demonstrated the effect of mastitis on the curd properties and on the general chemical characteristics of milk.

The freezing of milk, cream and condensed milk also has received considerable attention from this research worker. Particularly noteworthy are his studies during the recent war years on the freezing of fluid and condensed milk to ascertain the most desirable procedure to preserve the normal milk flavor and to prevent undesirable protein effects which normally occur in milk as the result of freezing.

During recent years he became interested in improving the nutritional value of evaporated milk and conducted fundamental research on the ascorbic acid content of this product. His work revealed that fortification of evaporated milk with ascorbic acid is feasible and under proper conditions ascorbic acid may be retained in this product for a considerable period of time.

This research worker also has been instrumental in developing and applying techniques for the analysis of milk and milk products. Among his contributions in this connection may be listed the colorimetric picric acid method for measuring lactose in milk, a modified Babcock test for determining the fat content of chocolate milk, a rapid drying method for determining the solids content of milk products by use of forced heated air, studies of methods of measuring the phosphatase activity in milk, and the application of a modified direct microscopic method for the bacterial analysis of milk.

In addition to his many personal contributions to dairy science, this year's nominee has long been known as an outstanding teacher and counselor of research men. He has directed the graduate program for many men who now hold responsible positions in industry and in college work and has done much to stimulate their thinking along research lines.

This year's nominee was born on September 20, 1896, in Philadelphia, Pennsylvania. He received both his undergraduate and graduate training at the Pennsylvania State College. Following the completion of his undergraduate work he spent

3 years as chemist with the Nestles Food Co. and 1 year as laboratory director for the Waddington Condensed Milk Co. He was instructor in dairying at the University of Maryland from 1922 to 1925. He accepted a position on the staff of the Pennsylvania State College and has remained on the staff of that institution since that time.

Principally because of his varied and valuable research in the fields of fluid and condensed milk during the past 27 years, but also because of his stimulating leadership and his training of men in Dairy Technology and his general contributions to the dairy industry the American Dairy Science Association Borden Award Committee for the Dairy Manufacturing Section has selected Francis Janney Doan, Professor of Dairy Manufacturing, the Pennsylvania State College, for the 1949 Award.

Mr. W. A. Wentworth of the Borden Company Foundation then presented Dr. Doan with a gold medal and a check for \$1,000.00.

BORDEN AWARD IN DAIRY PRODUCTION

G. M. Cairns, chairman of the Borden Award Committee for Production then was introduced and made the following statement:

The recipient of the Borden Award in Production for 1949, is George Herman Wise of the North Carolina State College, Raleigh, N. C. Dr. Wise was born and reared in South Carolina. He received his B.S. degree from Clemson Agricultural College in 1930, and his M.S. and Ph.D. degrees from the University of Minnesota in 1932 and 1936, respectively.

Dr. Wise has held the following positions since graduation from Clemson: Assistant, University of Minnesota 1934-36; Associate Dairy Husbandman, Clemson Agricultural College 1936-1944; Associate Professor of Dairy Husbandry at Kansas State College 1944-1947; Associate Professor of Dairy Husbandry and Research Assistant at Iowa State College 1947-1949 and since April 1949 Professor of Animal Industry at North Carolina State College, in charge of the nutrition section.

During his student days he was characterized by the thoroughness of his preparation in both study and research work. Since completing his graduate study he has continued to exhibit those same qualities in his research activities. An important contribution made by Dr. Wise was his series of comprehensive studies upon the physiology of gastric digestion in the calf. These include the various factors affecting the passage of liquids into the rumen of the calf and the changes in milk products that took place when milk was sham-fed. His more recent studies include the effect of the prepartum diet of the cow on the vitamin A and tocopherol content of colos-

trum and milk in the early post-colostrum period and upon the vitamin A storage in the new born calf. He received the American Feed Manufacturers' Association Award in 1948 for his series of publications on the latter work.

Dr. Wise has published more than 25 papers in the nutrition and physiology fields. He has been active in the Association, having served as Chairman of the Production Section in 1948 and is an Associate Editor of the JOURNAL OF DAIRY SCIENCE.



GEORGE HERMAN WISE

On behalf of the Committee on the Borden Award for Dairy Production, it is a pleasure to present Dr. George H. Wise to receive the Award.

Mr. W. A. Wentworth of the Borden Foundation then presented Dr. Wise with a gold medal and a check for \$1,000.

AMERICAN FEED MANUFACTURERS' AWARD

R. B. Becker, acting chairman of the Award Committee for the American Feed Manufacturers' Association, then was introduced and made the following statement:

Professor J. G. Archibald, chairman of the American Dairy Science Association committee to determine the recipient for the Award by the

American Feed Manufacturers Association regrets his inability to be present because of a conflicting engagement in the East, so it becomes my pleasure to represent Chairman Archibald on this occasion.

Seven persons were nominated for consideration this year. Your committee also canvassed the literature in the field of dairy cattle nutrition published during the two-year period 1947-48, as required by the rules. Over 50 papers were found eligible, all of which were evaluated carefully. The first draft reduced them to 23, and



THOMAS S. SUTTON

the decision narrowed finally to a group of six outstanding papers by one author and his co-workers.

These six papers have appeared in the JOURNAL OF DAIRY SCIENCE, one in 1947 and five in 1948. They dealt with comparisons of colostrum with normal milk as sources of vitamins and amino acids in the nutrition of dairy calves. The important and economic desirability of feeding as much colostrum as possible to young calves was established definitely by their investigations. The value of these findings to the dairy industry through healthier and more vigorous calves is obvious.

The worker who merited the award this year is widely known to nearly every member of this Association. He is an Ohioan by birth, scholastic

training and professional position. His career has been one of rapid promotion, from instructor in 1929 to head of Agricultural Biochemistry in 1948. He has been active in our Association, and served as editor of the JOURNAL OF DAIRY SCIENCE over the period 1938 to 1946.

By now, all of you must have recognized his identity. The committee is happy to present as candidate to the representative of the American Feed Manufacturers' Association for the 1949

Award, Dr. Thomas S. Sutton, of Ohio State University, Columbus, Ohio.

Dr. Record, it gives me great pleasure to serve in place of Chairman Archibald in presenting Dr. Thomas S. Sutton to you, as candidate for the 1949 Award.

P. R. Record, vice-chairman of the Nutritional Council of the American Feed Manufacturers' Association, then presented Dr. Sutton with a check for \$1,000.

NOTICE

Those wishing a copy of the material presented on the panel discussion on "The Job of Dairy Herd Improvement" that was held by the Production and Extension Sections at the recent meeting of the American Dairy Science Association may obtain this material in mimeographed

form by writing to the Editorial Department, Hoard's Dairyman, Fort Atkinson, Wisconsin. Tape recordings were made of the discussions and the resulting material has been edited by the members of the panel and mimeographed through the courtesy of Hoard's Dairyman.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

SEPTEMBER, 1949

NUMBER 9

A MODIFIED PHOSPHATASE TEST FOR CHEESE¹

F. V. KOSIKOWSKY AND A. C. DAHLBERG

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

In a recent paper (5) the authors presented a new principle and technic for the elimination of interfering substances in the Kay-Graham phosphatase test on Cheddar cheese. Additional research indicated that the trichloroacetic acid technic could be simplified and improved further, and made into a phosphatase method for cheese possessing some distinct advantages over present methods. For example, the use of sodium barbitol was found unsatisfactory for cheese, as the optimum pH for phenol production could not be attained. As a result, a sodium carbonate-bicarbonate buffer (6) was substituted with highly satisfactory results. Other changes included the use of small phenol extraction flasks having a lower chamber capacity of 5 ml. and the combining of the trichloroacetic acid and hydrochloric acid as the precipitating agent. A significant observation was that the BQC color reagent could be used interchangeably with the Folin-Ciocalteu color reagent in this new method.

With these considerations in mind, a more detailed account of this modification as a quantitative method is being presented, as well as the results from a series of experiments on Cheddar cheese showing the precision with which this method distinguishes raw from pasteurized milk cheeses.

PROCEDURE

Reagents²

1. *Carbonate-bicarbonate buffer substrate.* Weigh 11.5 g. C.P. sodium carbonate, anhydrous; 10.15 g. C.P. sodium bicarbonate, anhydrous; and 1.09 g. pure disodium phenyl phosphate, dissolve in distilled water and make up to 1.1. The pH will be 9.80. When not used fresh, add 10 ml. U.S.P. chloroform. This buffer should be tested after storage to assure freedom from phenol.

2. *Trichloroacetic-hydrochloric acid precipitant (12.5 per cent trichloroacetic acid plus 18 per cent HCl).* Make 25 g. C.P. trichloroacetic acid (crystal) to 50 ml. with distilled water, add 50 ml. concentrated C.P. HCl (approximately 36

Received for publication March 9, 1949.

¹ This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Catherine Verwoert Work and Mr. Allan Levanthal for their aid in making many of the chemical analyses, and to Professor W. E. Ayres for his aid in the manufacture of several lots of cheese.

² Keep all reagent bottles and color standards tightly stoppered and in a cool place.

per cent) and stir well. The resulting liquid is slightly yellow and can be held for a number of days, but fresh solution is preferable.

3. *Ether*. U.S.P. grade.

4. *Sodium carbonate solution (4 per cent)*. Dissolve 40 g. C.P. anhydrous sodium carbonate in distilled water and make up to 1 l. It is best to make this solution fresh, but it may be held well-stoppered in the refrigerator for a day or two.

5. *Folin-Ciocalteu reagent*.³ It is prepared as stock solution according to A.O.A.C. (1) procedure for use with milk. The stock reagent should be diluted with two parts of water just before using.

6. *Permanent Kay-Graham color standards and colorimeter*. For producing permanent color standards refer to A.O.A.C. (1) or to Gilcreas and Davis (3). The color standards are the same as for milk.

Luximeter (no. 5) can be purchased from General Electric Co., Schenectady, New York.

The Method. One-half g. of a representative sample of ground cheese was placed in a 25 × 150 mm. test tube and mashed well with a glass rod. Following this, 1 ml. of warm (40° C.) carbonate-bicarbonate buffer substrate was added and the cheese was stirred into a paste. Then 9 ml. more of the buffer substrate and four drops of U.S.P. chloroform were stirred into the tube. A piece of parchment paper was fitted over the rod and tube and held in place by a rubber band. The tube next was incubated at 32 to 37° C. for 18 to 24 hours. After incubation, 1 ml. of the trichloroacetic-hydrochloric acid precipitant was added gently to the tube. The pipet may be filled by immersion rather than by suction. The resulting precipitate was filtered off through Whatman no. 42 paper (11 cm.)

Five ml. of the clear filtrate then were pipetted into a small-sized Mojonnier type extraction flask (fig. 1). Next, 15 ml. of ethyl ether at 10 to 20° C. were added and the flask stoppered.⁴ The flask then was inverted slowly ten times. For this purpose a special combined holder and shaker may be used (fig. 1). A clear ether layer extending to the neck of the flask developed after about 10 seconds of standing and was poured off into a 25 × 150 mm. test tube containing 7 ml. of 4 per cent Na₂CO₃ solution. The ether then was boiled off, in about 4 minutes by placing the tube in a beaker of hot water (150 to 160° F.) or on a steam-heated water bath.

After the ether was removed completely, 2 ml. of diluted Folin-Ciocalteu reagent (two parts water to one part stock reagent) were added and shaken. The mixture was placed in boiling water for 5 minutes, cooled to room temperature and filtered. The colored filtrate was compared to color standards or read in a Luximeter. Tentatively, values greater than 0.02 mg. phenol per 0.5 g. cheese indicate cheese made from underpasteurized or raw milk.

Use of 2-6-dibromoquinone-chloramide (BQC) instead of Folin-Ciocalteu reagent. Four drops of BQC solutions can be added in place of the Folin-Ciocalteu

³ May also be purchased from Will Corporation, Rochester, N. Y. as stock solution.

⁴ Extraction flasks can be purchased with either plain necks or ground glass stoppers. If cork stoppers are used they should be covered with clean tin foil.

reagent with very satisfactory results. The color is allowed to develop for 15 minutes and then compared against suitable carbonate-bicarbonate color standards. The ability to use BQC or Folin-Ciocalteu reagent gives greater flexibility to the method in that it would be easy to get checks on the results by splitting the carbonate solution containing the phenol and adding one of these indicators to each portion. In addition, using BQC in this method provides some advantages over other methods using BQC. Apparently very little or no protein or protein products are present in the final carbonate solution, so there would be no interfering yellow color-producing compounds. For this same reason, butyl alcohol may be used for extraction without encountering an emulsion after shaking, thus doing away with all centrifuging which is required at this point using other methods. Add 5 ml. of N-butyl alcohol, shaking the tube ten times, wait for 1 minute, and read colors at eye level or in a colorimeter.

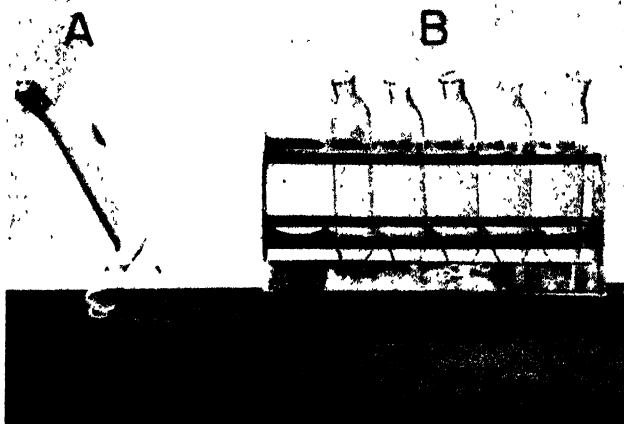


FIG. 1. A--Small phenol extraction flask. Manufactured by Will Corporation, Rochester, N. Y., and Mojonnier Bros. Co., Chicago, Ill. Extraction flasks with ground glass necks and stoppers also obtainable from Will Corporation, Rochester, N. Y. B--Extraction flask holder (use optional). Manufactured by Mojonnier Bros. Co., Chicago, Ill. Holder also can be made in laboratory using either sheet metal or wood.

Sensitivity of method with Folin-Ciocalteu and BQC reagent. Three lots of fresh raw milk were obtained on three different days from the Cornell University herd. One lot was divided into 350-lb. portions, heated to 143 or 145° F. in glass-lined vats and different portions held at this temperature for varying holding periods. Another lot of milk was divided into portions which were heated to various temperatures in the vicinity of 143° F., but all portions were held for 30 minutes. A third lot of milk was pasteurized properly at 143° F. for 30 minutes, but to separate portions were added different amounts of raw milk. The milks were tested by the standard laboratory Kay-Graham test and gave values corresponding to those expected from the heat treatment of the milk.

A total of 16 American Cheddar cheeses, weighing about 35 lb. each, were made from the above milks and were ripened at 50° F. for 6 months. These cheeses then were tested for phosphatase activity and sensitivity by the modified method using both the Folin-Ciocalteu and the BQC reagents.

Results using the Folin-Ciocalteu reagent are presented in table 1. When the milk was pasteurized properly the phosphatase values on the cheeses were less than 0.01 mg. phenol per 0.5 g. cheese. At no time in the analyses of a large

TABLE 1

The sensitivity of the modified phosphatase test on cheddar cheese ripened 6 mo. at 50° C. (incubated 20 hr. at 35° C.)

Heat treatment of cheese milk		Phosphatase values of cheese	
		Using the Folin-Ciocalteu color reagent ^a	Using BQC color reagent ^b
(° C.)	(min.)	(mg. phenol/0.5 g. cheese)	(γ phenol/0.5 g. cheese)
145	30	0.003	0.5
143	30	0.006	1.0
143	25	0.034	12.0
143	20	0.033	14.0
143	10	0.128	> 40.0
143	0	0.654	> 40.0
143	30	0.007	1.0
141	30	0.107	> 40.0
139	30	0.227	> 40.0
137	30	0.654	> 40.0
143 - 30 + No Raw		0.003	1.0
143 - 30 + 0.1% Raw		0.032	9.0
143 - 30 + 0.2% Raw		0.048	18.0
143 - 30 + 0.4% Raw		0.064	> 40.0
143 - 30 + 0.7% Raw		0.115	> 40.0
143 - 30 + 1.0% Raw		0.183	> 40.0

^a A reading of 0.05 mg. phenol/0.5 g. cheese indicates a very deep blue color using the Folin-Ciocalteu reagent, as the units (mg.) are 1,000 times greater than the units (γ) expressed when using the BQC color reagent.

^b 2-6-dibromoquinone-chloramide. Dissolved 50 mg. in 10 ml. of methyl or ethyl alcohol.

number of Cheddar cheeses made from properly pasteurized milk have the phosphatase values extended beyond 0.02 mg. phenol per 0.5 g. cheese. On the other hand, a change of from 30 to 25 minutes of holding the cheese milk at 143° F. produced a phosphatase value on the cheese greater than 0.02 mg. phenol per 0.5 g. cheese. Similarly, when the temperature of heating the cheese milk was reduced from 143 to 141° F. and held for 30 minutes, a phosphatase value of 0.11 mg. phenol per 0.5 g. cheese was recorded. In the case of raw milk contamination, and addition of 0.1 per cent raw milk manifested itself as a phosphatase test greater than 0.03 mg. phenol per 0.5 g. cheese.

Data showing how well results obtained on Cheddar cheeses over one year old compared to those obtained by the method of Sanders and Sager (7) are shown in table 2.

TABLE 2
The phosphate test of cheddar cheese aged over 1 yr. and made from pasteurized milk to which raw milk had been added

Milk	Extraction procedure Kosikowsky-Dahlberg ^a	Sanders-Sager ^b		
		Direct readings ^c	(γ phenol/0.25 g.) (interpretation)	Butyl alcohol readings ^d
Pasteurized (143° F., 30 min.)			(γ phenol/0.25 g.) (interpretation)	(γ phenol/0.25 g.) (interpretation)
Pasteurized + 0.1% raw	0.008	Pasteurized	0.0	0.3
Pasteurized + 0.2% raw	0.018	Pasteurized	0.0	0.3
Pasteurized + 0.3% raw	0.031	Underpasteurized	0.5	0.8
Pasteurized + 0.7% raw	0.050	Underpasteurized	1.1	2.0
Pasteurized + 1.0% raw	0.086	Underpasteurized	2.2	3.0
	0.131	Underpasteurized	3.8	4.1

^a More than 0.02 mg. indicates underpasteurization (Folin-Ciocalteu reagent).

^b More than 3 γ indicates underpasteurization.

^c Color in blanks, pinkish-yellow; in test, yellowish-blue; blank reading 0.

^d Color in blanks, pinkish-yellow; in test, bluish-green; blank reading of 0.3 subtracted from results on cheese.

When BQC color reagent was used in place of the Folin-Ciocalteu color reagent the sensitivity was equally as good and the value of phenol which apparently divided the cheeses made from properly pasteurized milk from those made from improperly pasteurized milks was about 5.0 γ phenol per 0.5 g. cheese.

Amount of phenol obtained from a second extraction. Phenol is somewhat soluble in water at 20° C., although in ether it is extremely soluble. Possibly an appreciable amount of free phenol never was extracted from the aqueous acid solution in one extraction. That this is not the case was verified experimentally. A number of incubated cheese-buffer substrate solutions, after precipitation with trichloroacetic-hydrochloric acid, were extracted with two 15 ml. portions of ethyl ether. About 92 to 96 per cent of the free phenol was extracted the first time. The amounts obtained from a second extraction would not affect materially the initial values, as they were very small and would, on the whole, remain fairly constant for pasteurized milk cheese.

Effectiveness of the trichloroacetic-hydrochloric acid precipitating agent. Cheddar cheeses ranging in age from 2 days to 11 years, as well as 14 other varieties of cheese of varying age, were mixed in 0.5 g. lots with 10-ml. quantities of carbonate-bicarbonate buffer substrate, well-emulsified, and then 1 ml. of trichloroacetic-hydrochloric acid reagent was added to each tube. This precipitating agent would precipitate very satisfactorily cheeses of all ages and varieties without any further adjustment. This was shown by the clear filtrates which were produced.

DISCUSSION

A technic to eliminate interfering substances in the Kay-Graham phosphatase procedure for ripened cheese as proposed earlier by Kosikowsky and Dahlberg (5) now is presented in more detail and in simplified form as a laboratory phosphatase method for all cheese. This method possesses advantages. It produces clear filtrates devoid of interfering substances which give clear blue colors matching standards exactly, thus providing for greater accuracy. It has great flexibility, as either the Folin-Ciocalteu or the BQC color reagent may be used simply by substituting one for the other in the last step. The sodium carbonate-sodium bicarbonate buffer and the trichloroacetic-hydrochloroacetic acid precipitating agent in this method, although more study may be necessary, may be used for any variety of natural or process cheese or cheese product of any age without adjusting either the buffer or the precipitating agent to care for differences in the buffer capacities of the cheeses. The buffer substrate and cheese need not be heated after incubation to destroy the phosphatase. Therefore, there is less tendency to decompose the disodium phenyl phosphate. When using butyl alcohol for BQC color extraction purposes, one can shake the tube vigorously without forming a permanent emulsion. The phosphates and citrates in process cheese will not affect the final results when using the carbonate-bicarbonate buffer substrate, as shown by Kosikowsky and Dahlberg (6). Finally, few reagents are required and a number of these, including the Folin-Ciocalteu reagent, may be purchased as stock solutions from chemical firms.

Although this method no longer closely resembles that of the standard Kay-Graham method for milk (4), it nevertheless possesses some of the elements and the basic principles. An inclusion of this test for cheese would make the Kay-Graham test (4) more universally applicable. This is important, as the standard Kay-Graham test is a proved basic test for milk and other dairy products. Preliminary studies also indicate this modified method will work well on chocolate milks and ice creams.

The fact that an 18- to 24-hour incubation period is recommended should not prove to be any disadvantage for a laboratory test dealing with cheese. The longer incubation period would allow a more orderly and efficient use of laboratory time, as a large number of samples could be placed in the incubator in the afternoon and then could be run the next morning. This idea has been substantiated by an informal survey of men in charge of research for cheese companies and in charge of state testing laboratories. If speed is essential, an extraction method using shorter incubation periods of 1 or 4 hours at 37° C., using BQC color indicator, might be developed.

Actually, using the method in its modified form does not entail much more operating time than that involved in the standard Kay-Graham laboratory method for milk (4) nor is it more expensive. As each step can be organized to fit in well with the next without having to consider any time element beyond which the test would not function properly, a large number of samples may be run during the day. From the time of adding the precipitating agent after incubation, two analysts completed over 25 individual samples in 1 hour.

When the small phenol extraction flasks are not available, equally good results can be obtained with standard-size Mojonnier fat extraction flasks. Add 5 ml. of filtrate to the flask and enough distilled water (25° C.) to bring to bottom of neck. Add 25 ml. of ethyl ether and shake ten times. Proceed thereafter as with regular modified method. Where extraction flasks are unavailable, small separatory funnels may be substituted as a temporary measure. With these funnels 5 ml. of filtrate can be shaken ten times with 15 ml. of ethyl ether; the filtrate then is taken off through the bottom and the ether is poured off through the top.

The results have been expressed empirically as mg. phenol per 0.5 g. of cheese to agree approximately with the Kay-Graham method (4). The data may be calculated readily to exact quantitative expression of results to agree with the expression used by Sanders and Sager (7). As 0.5 g. of cheese was diluted to 11.5 ml., from which 5 ml. of filtrate were used, the results obtained as mg. phenol could be multiplied by 1.15 to give quantitatively the mg. phenol per 0.25 g. cheese. If then multiplied by 1,000, the results are in γ phenol per 0.25 g. cheese.

Two factors favor greater accuracy by this modified procedure than by the Sanders and Sager method (7), which is the only other method applicable to cheese. The color in the present procedure has no interfering off-colors and the pH, the buffer and the longer time of incubation induce maximum enzyme activity. The greater sensitivity is indicated by the standard or criterion for

pasteurization. In the Sanders and Sager method (7) pasteurization is indicated by 3 γ or less of phenol per 0.25 g. cheese. In the procedure herein presented using Folin's reagent, 0.02 mg. phenol per 0.5 g. cheese or less represents pasteurization. This is 23 γ phenol per 0.25 g. cheese, or about eight times the quantity of phosphatase activity shown by the Sanders and Sager method (7). The greater sensitivity also is shown by the effect of known amounts of raw milk upon the results. In collaborative tests on cheese, Gilcreas (2) found it was difficult to detect 0.3 per cent of raw milk added to pasteurized milk using the Sanders-Sager method as nine of the fifteen collaborators found such cheese to be made from pasteurized milk. The values varied from 1 to 7 and averaged 3.11 γ phenol per 0.25 g. cheese, which is only 0.1 γ above the criterion for pasteurization. The modified Kay-Graham extraction method in the present study gave results over twice the criterion for pasteurization with only 0.2 per cent of raw milk. Again Gilcreas (2) reported data for the 15 collaborators on cheese made from pasteurized milk containing 1 per cent of raw milk which varied from 4 to 11 γ phenol per 0.25 g. cheese, and the average value was 2.5 times the critical value for pasteurization using the Sanders-Sager method. The data herein presented for the same raw milk contamination show values in cheese of 9 times the critical value for pasteurization.

PRECAUTIONS

Precautions involved in the use of this method should be pointed out. In rare cases a temporary emulsion may be formed in the upper chamber of the extraction flask during extraction (the narrow emulsified layer which may be formed between the ether and aqueous layer in the neck of the flask is considered normal). This condition may be caused by very excessive agitation or the use of too porous filter paper, causing turbid filtrates. To remove this temporary emulsion, place the extraction flask, with the cork loosely stoppered, under the hot water tap (140 to 160° F.) for 10 to 20 seconds, shake the flask briefly and then cool quickly under the cold water tap.

In boiling off the ether, fire safety rules should be followed. The ether should be boiled off completely to make a satisfactory test. This can be seen easily by visual inspection or by carefully shaking the tube to detect foaming. In some cases the ether reagent may show a slight trace of phenol. This can be detected and compensated for by conducting a reagent blank test on all reagents and going through all the steps in the process, including incubation.

Although control samples on the cheese consistently have given very low values, control tests are advisable where it is suspected phenol may have formed during ripening. To do this heat the sample of cheese to 170° F. for 5 minutes in a test tube and cool, take 0.5 g. of the cooled sample, mix it with 10 ml. of carbonate-bicarbonate buffer substrate and conduct a regular test on it.

The extraction flasks are rinsed out easily between samples with lukewarm tap water. These flasks need not be dry, but as the smaller extraction flasks are calibrated for 5 ml. there should not be too much free water in the lower chamber.

SUMMARY

A phosphatase test for cheese is presented in detail with data showing its sensitivity. This method possesses a number of advantages. It is very accurate, as the final filtrates produced in this modification were clear and devoid of interfering substances. Conditions of incubation promote maximum phosphatase activity. The Folin-Ciocalteu color reagent and the BQC color reagent can be used interchangeably with proper color standards. Apparently, any natural or process cheese or cheese product can be tested without making adjustments in the buffer substrate or precipitating agent.

In the new method the cheese sample is incubated with a sodium carbonate-bicarbonate buffer substrate at pH 9.55 for 18 to 24 hours at 32 to 37° C. This solution then is precipitated by a trichloroacetic-hydrochloric acid reagent and some of the clear filtrate is placed in either a standard size or a small Mojonnier type extraction flask. Ethyl ether is used as the extracting agent but later is boiled off from an alkaline solution leaving the phenol behind. The phenol in this alkali solution then is determined colorimetrically by adding either the Folin-Ciocalteu or the BQC color reagent and comparing the developed blue colors against proper color standards.

The sensitivity of this method is high. Tentatively, any value above 0.02 mg. phenol per 0.5 g. cheese, using the Folin-Ciocalteu reagent, or any value over 5.0 γ phenol per 0.5 g. cheese, using the BQC reagent, is considered to indicate cheese made from raw milk, improperly pasteurized milk or pasteurized milk contaminated with raw milk.

REFERENCES

- (1) American Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*. 5th Edition. Pp. 319-320. Washington, D. C. 1945.
- (2) GILCREAS, F. W. Report on Tests for Pasteurization of Dairy Products. The Phosphatase Test in the Examination of Hard Cheese. *J. Assoc. Offic. Agr. Chemists*, **30**: 422-430. 1947.
- (3) GILCREAS, F. W., AND DAVIS, W. S. Investigation of the Amylase and Phosphatase Tests as an Indication of Pasteurization. 24th Ann. Rept. Intern. Assoc. Milk Sanitarians, pp. 15-32. 1936.
- (4) KAY, H. D., AND GRAHAM, W. R., JR. The Phosphatase Test for Pasteurized Milk. *J. Dairy Research*, **6**: 191-203. 1935.
- (5) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Elimination of Interfering Substances in the Kay and Graham Phosphatase Test when Used for Hard Ripened Cheese. *J. Dairy Sci.*, **31**: 561-567. 1948.
- (6) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. Application of a Sodium Carbonate-Sodium Bicarbonate Buffer Substrate in the Phosphatase Test for Cheese. *J. Dairy Sci.* In press.
- (7) SANDERS, G. P., AND SAGER, O. S. Phosphatase Test for Various Dairy Products. *J. Dairy Sci.*, **31**: 909-920. 1947.

APPLICATION OF A SODIUM CARBONATE-BICARBONATE BUFFER IN THE PHOSPHATASE TEST FOR CHEESE¹

F. V. KOSIKOWSKY AND A. C. DAHLBERG
Department of Dairy Industry, Cornell University, Ithaca, N. Y.

In the past a number of different buffers have been used in the buffer substrate when conducting the phosphatase test. Kay and Graham (6) have successfully used sodium barbitol in their test for milk, Aschaffenburg and Neave (1) have suggested the use of sodium carbonate, whereas Scharer (10) has used a borate buffer.

As far as cheese was concerned, Caulfield and Martin (2), Lambert (8) and Sanders and Sager (9) all have stressed the importance of a proper buffer as the cheese, because of its different buffering capacity, exerts a decided effect on the pH of the buffer substrate. If a buffer were unable to maintain the proper pH for optimum phenol production low values would be obtained. Although Sanders and Sager (9) appear to use a very good modified borate buffer, it still is necessary in their test to make adjustments for different aged cheeses or cheeses of different varieties. During a recent study (7) on the elimination of interfering substances in the phosphatase test, it was noted that the sodium barbitol buffer used was affected greatly by cheeses of different age or variety and that additions of cheese invariably would carry the pH of the cheese-buffer substrate solution much lower than that recommended by earlier investigators for optimum phenol production by the alkaline phosphatases. As a result, it was decided to abandon the sodium barbitol buffer and substitute one which would maintain the pH near its optimum for phenol production.

A study of several buffers pointed to a sodium carbonate-sodium bicarbonate combination as showing the most promise. This buffer had been studied earlier on the alkaline phosphatase systems by Fischer (4), Schwarz and Fischer (11), Horwitz (5) and Delory and King (3). The latter investigators stated that the carbonate-bicarbonate buffer can be held for as long as 6 months over a pH range of from 8.8 to 10.6 at 37° C. in well-stoppered wax bottles without any demonstrable change in pH. As this buffer covered a region of higher pH than did the sodium barbitol buffer, a study was made to determine the optimum pH for phenol production with this buffer and also its effectiveness in maintaining the optimum pH in the presence of cheese. The concentration of carbonate in the buffer used for this experimental work was much greater than that used in the carbonate buffer of Delory and Kling (3) and others (4, 11).

METHODS

To study phenol production at varying pH values, the trichloroacetic acid technic for the Kay-Graham test recently presented by Kosikowsky and Dahlberg (7) was used with the exception that the sodium carbonate-bicarbonate buffer was

Received for publication March 9, 1949.

¹ This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Catherine Verwoert Work for her aid in making many of the chemical analyses.

substituted for sodium barbitol in the buffer substrate. A series of buffer substrates was used with a wide range of pH. These differences were obtained by varying the relative proportion of sodium carbonate to sodium bicarbonate while keeping the carbonate concentration constant in each buffer substrate solution. One-half g. portions of a sample of Cheddar cheese were incubated for 20 hours at 35° C. in 10 ml. portions of the different buffer substrates. The following day quantitative phenol determinations were made.

RESULTS

Optimum pH for phenol production. Data obtained using the new sodium carbonate-bicarbonate buffer are shown in table 1. The optimum pH for the

TABLE 1

The optimum pH for phosphatase activity on cheese and milk using the carbonate-bicarbonate buffer substrate over 18-hour incubation at 32-35° C.

pH of buffer substrate plus milk	Phenol values (mg. phenol/0.5 g. milk)	pH buffer substrate plus cheese	Phenol values (mg. phenol/0.5 g. cheese)
11.04	0.007	10.55	0.005
10.32	0.009	10.25	0.016
9.97	0.035	10.05	0.028
9.83	0.045	9.95	0.049
9.71	0.056	9.79	0.073
9.64	0.062	9.63	0.115
9.47	0.051	9.47	0.111
9.22	0.042	9.32	0.068
8.95	0.023	9.05	0.062
		8.68	0.052
		7.93	0.019

production of phenol with cheese was approximately 9.55, while the optimum range roughly extended from about pH 9.40 to 9.70. Optimum pH data obtained with a sample of milk were very similar.

Effectiveness of the carbonate-bicarbonate buffer in maintaining the optimum pH range. The carbonate-bicarbonate buffer substrate used in this portion of the study was the same as that which was found to produce the optimum pH. This solution contained 11.5 g. anhydrous sodium carbonate, 10.15 g. sodium bicarbonate and 1.09 g. disodium phenyl phosphate made up to 11. with water. This solution had a pH of 9.8 measured at 25° C.

Cheddar cheeses ranging in age from 2 days to 11 years, as well as samples from 14 other varieties of cheese of varying age, were mixed in 0.5 g. lots with 10-ml. quantities of the carbonate-bicarbonate buffer substrate. In addition, fluid milk and fluid milk products, using 1 ml. per 10 ml. buffer, also were tested. Measurement of pH at 25° C. was conducted a few minutes after mixing and again after incubation for 24 hours at 35° C. Data obtained are shown in table 2.

Addition of 0.5 g. portions of cheese reduced the pH, on the average, to about 9.55 or a drop of 0.25 pH unit. For all the cheeses tested, the pH change in the buffer substrate did not extend beyond the optimum pH range of 9.45 to 9.70

TABLE 2

Effectiveness of carbonate-bicarbonate buffer in controlling the pH of cheese and other dairy products
(optimum pH at 35° C. using carbonate-bicarbonate buffer = 9.55 ± .15)

Dairy Product	Age	pH Range ^c
12 Cheddar cheeses	1 to 132 mos.	9.40-9.68
19 Other cheese varieties ^a	Unknown	9.49-9.65
Other dairy products ^b	Fresh	9.61-9.71

^a Process Limburger, process gruyere, cream, pimento cream, Swiss, Limburger, Liederkranz, Chantelle, Snappy club, Kaukauna club, Sharp process, Munsko, d'Oka, Edam, Blue, Romana, Cottage.

^b Milk, Heavy cream, Chocolate milk, Bottle milk, Sour cream, Vanilla ice cream mix, Chocolate ice cream mix.

^c All pH measurements in this study made with Beckman pH meter, laboratory model G.

after incubation for 24 hours at 35° C. Where milk and other fresh dairy products were tested the pH ranged from 9.5 to 9.7.

Effect of phosphates and citrates upon phosphatase activity in the presence of the carbonate-bicarbonate buffer substrate. Sanders and Sager (9) have stated that excess phosphates and citrates, as normally found in process cheese, may inhibit the action of phosphatases but that in the presence of a borate buffer this inhibiting effect does not occur. With a view towards applying the carbonate-bicarbonate buffer substrate to eventual use on process cheese, an investigation was made of the significance of excess citrate and phosphate emulsifying salts in natural Cheddar cheese. To a number of Cheddar cheeses, 3 per cent quantities of disodium phosphate or sodium citrate were added and the trichloroacetic acid technic, using a 20-hour incubation period, at 35° C. was conducted on the cheese. Table 3 shows that excess amounts of phosphates and citrates exert no effect upon

TABLE 3

The phosphatase values of cheddar cheese with and without added stabilizing salts, using the trichloroacetic technic on the K₂Graham method with a sodium carbonate-bicarbonate buffer

Cheese	Phosphatase values of cheese		
	Natural cheddar cheese no added salt	Natural cheddar cheese plus 3% NaH ₂ PO ₄	Natural cheddar cheese plus 3% sodium citrate
	(mg. phenol/0.5 g. cheese)		
1	0.003	0.009	0.006
2	0.003	0.010	0.013
3	0.032	0.038	0.038
4	0.048	0.048	0.048
5	0.064	0.083	0.093
6	0.115	0.118	0.135
7	0.183	0.181	0.158
8	0.654	0.676	0.632

phenol production in the presence of carbonate buffer substrate. This would mean that this technic could be used with process cheese.

DISCUSSION

The use of a sodium carbonate-bicarbonate buffer in the modified trichloroacetic acid phosphatase test for cheese appears to show promise. This buffer was used in greater concentration than by previous investigators. It has the advantages of being very inexpensive, very soluble and easily obtainable. At the concentration and pH at which it will be employed it is stable, according to Delory and King (3). This recently was verified in this work. In addition, this buffer is strikingly effective in maintaining optimum pH with cheeses of wide buffer capacities and in its presence excess amounts of phosphate and citrate salts exert no noticeable effect upon phosphatase activity. This buffer shows no noticeable inhibition of phosphatase activity in the 18- to 24-hour test at around pH 9.6, using the trichloroacetic technic of the Kay-Graham method.

SUMMARY

A sodium carbonate-bicarbonate buffer solution containing 11.50 g. sodium carbonate and 10.15 g. sodium bicarbonate per liter of solution has been found to be highly effective in maintaining optimum pH for phosphatase activity in the trichloroacetic acid Kay-Graham 24-hour phosphatase test for cheese.

Optimum pH for phosphatase activity was maintained with the single sodium carbonate-bicarbonate buffer upon the addition of a great variety of dairy products, including very aged hard cheeses.

The optimum pH for phosphatase activity during incubation for 24 hours at 35° C. with a carbonate-bicarbonate buffer substrate was 9.55 ± 0.15 .

REFERENCES

- (1) ASCHAFFENBURG, R. AND NEAVE, F. K. A Rapid Phosphatase Test. *J. Dairy Research*, 10: 485-497. 1939.
- (2) CAUFIELD, W. J., AND MARTIN, W. H. Determination of Improper Pasteurization by Applying the New York City Field Phosphatase to Cheddar Cheese. *J. Dairy Sci.*, 28: 155-160. 1945.
- (3) DELORY, G. E. AND KING, E. J. A Sodium Carbonate-bicarbonate Buffer for Alkaline Phosphatases. *Biochem. J.*, 39: 245. 1945.
- (4) FISCHER, O. The Phosphatase Reaction as a Test to Indicate Prolonged and Flash-heating of Milk. *Deut. Milkeri-Ztg.*, 63: 520-521. 1942. (Chem. Abstracts, 38: 4047. 1944.)
- (5) HORWITZ, W. A Modified Scharer Phosphatase Test for Milk and Soft Cheeses. *J. Assoc. Offic. Agr. Chemists*, 29: 129-165. 1946.
- (6) KAY, H. D., AND GRAHAM, W. R., JR. The Phosphatase Test for Pasteurized Milk. *J. Dairy Research*, 6: 191-203. 1935.
- (7) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Elimination of Interfering Substances in the Kay-Graham Phosphatase Test when Used for Hard Ripened Cheese. *J. Dairy Sci.*, 31: 561-567. 1948.
- (8) LAMBERT, L. The Phosphatase Test and Its Application to Cheese. *J. Dairy Sci.*, 10: 751-757. 1945.
- (9) SANDERS, G. P. AND SAGER, O. S. Present Status of the Phosphatase Test. *J. Milk and Food Technol.*, 11: 67-75. 1948.
- (10) SCHARER, H. Application of the Rapid Phosphatase Test to Dairy By-products. *J. Milk Technol.*, 2: 16-20. 1939.
- (11) SCHWARZ, G., AND FISCHER, O. Über den Nachweis der Kurzeithitzung der Milch mit Hilfe der Phosphatasereaktion. *Die Milchwissenschaft*, 3: 41-45. 1948.

THE UTILIZATION OF FETAL STORES OF VITAMIN A BY THE NEWBORN CALF¹

H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON AND L. D. MATTERSON
Animal Industries Department, Storrs Agricultural Experiment Station, Storrs, Connecticut

The liver storage of vitamin A in the newborn calf can be increased by dietary means (6, 10, 16, 18). Whether this increased liver storage and other fetal stores of vitamin A can be utilized by the neonatal calf has not been demonstrated (14, 16).

The purpose of this study was to determine whether the fetal storage of vitamin A could be utilized in the young dairy calf, as indicated by blood plasma levels and liver storage of vitamin A when a vitamin A-free ration is fed.

EXPERIMENTAL

Nine male calves from Ayrshire, Guernsey and Holstein dams born in the University of Connecticut herd from February to June, 1948, were used in this experiment. The dams of these calves received the same basal ration fed on the basis of liveweight for 8 weeks prior to the calculated parturition date. This consisted, per 100 lb. liveweight, of 1 lb. of U. S. no. 2 alfalfa hay, 3 lb. of well-matured corn silage and 1 lb. of grain mixture consisting largely of cereal grains and containing approximately 13.5 per cent crude protein. The hay, silage and grain contained on an average of 3.87, 1.06 and 0.15 mg. of carotene per lb., respectively, as determined by the method of Moore and Ely (9) as modified by Nelson *et al.* (12). Six of the dams of these calves received daily 1 million U.S.P. units of vitamin A in the form of shark liver oil containing 25 per cent by weight of crude soybean lecithin² for 30 days prior to the calculated parturition date. This oil contained 54,440 U.S.P. units of vitamin A per gram, as assayed spectrophotometrically against the U.S.P. vitamin A reference standard (vitamin A acetate in cottonseed oil).

The newborn calves were not allowed to nurse but were removed immediately to a separate portion of the barn. There they received 3 lb. of reconstituted skim milk two times daily. The skim milk fed contained no detectable carotene or vitamin A, as determined by the method of Boyer *et al.* (1).

Venous blood samples were drawn from the jugular vein each day between 7 and 8 A.M., citrated, cooled to 4° C., and centrifuged; the carotene and the vitamin A contents of the plasma were determined immediately. On the tenth morning, the calves were slaughtered. The entire liver was removed and cooled at 4° C. The whole liver was nacerated in a Waring blender and analyses run on aliquot samples within 48 hours. In several cases the livers were frozen and held at -18° C. for a period not exceeding 2 weeks until analyses could be made.

Received for publication March 31, 1949.

¹ This work was supported in part by the Big Y-Foundation, Norwich, Conn., and Chas. M. Cox Co., Boston, Mass.

² This oil generously was supplied by Melvin Hochberg of the Nopeco Chemical Co., Harrison N. J.

The carotene and vitamin A contents of the plasma were determined by the method of Kimble (8) and that of the liver by the method of Davies (2). Both determinations were made with an Evelyn photoelectric macrocolorimeter which had been standardized previously with crystalline β -carotene in petroleum ether (b.p. 30–60° C.) and crystalline vitamin A alcohol in chloroform.

The data were treated statistically by analysis of variance essentially as outlined by Snedecor (15).

RESULTS

Data on the carotene and vitamin A content of plasma for individual calves are given in table 1. The carotene content of the plasma of calves from dams re-

TABLE 1

The effect of parturition supplementation of vitamin A on blood plasma levels of carotene and vitamin A in newborn calves fed reconstituted skim milk

Calf no.	Birth	Age (d.)								
		2	3	4	5	6	7	8	9	10
(γ carotene/100 ml.)										
Basal dams										
1	3	2	2	2	1	0	0	2	2	1
2	2	1	2	6	1	2	2	2	2	3
3	3	3	2	2	2	2	2	2	3	3
\bar{X}	2.7	2.0	2.0	3.3	1.3	1.3	1.3	2.0	2.3	2.3
Basal + vitamin A dams										
4	1	0	0	0	1	1	0	1	0	0
5	1	0	0	0	1	1	0	1	1	1
6	1	2	0	0	0	0	1	1	1	1
7	1	1	1	1	0	1	1	1	2	1
8	2	2	1	2	2	2	2	2	2	1
9	3	2	2	1	1	1	1	1	0	2
\bar{X}	1.5	1.2	0.7	0.7	0.8	1.0	0.8	1.2	1.0	1.0
(γ vitamin A/100 ml.)										
Basal dams										
1	4.9	3.8	5.3	2.4	4.9	1.8	1.4	1.4	1.3	1.4
2	4.4	8.2	1.4	2.5	1.4	2.1	4.4	4.3	3.8	2.6
3	4.2	3.0	0.8	1.7	0.5	0.8	0.8	0.6	0.5	0.8
\bar{X}	4.50	5.00	2.50	2.20	2.27	1.57	2.20	2.10	1.87	1.60
Basal + vitamin A dams										
4	5.2	6.8	9.7	9.2	9.9	11.1	6.2	8.8	7.0	7.0
5	7.0	5.7	8.8	8.8	8.3	9.0	5.3	7.8	6.5	7.5
6	13.5	9.2	8.3	7.5	9.9	12.3	12.8	13.5	14.6	12.6
7	9.9	11.3	10.2	9.6	11.6	13.8	14.3	6.2	17.3	17.0
8	11.1	10.1	9.6	6.0	6.0	11.1	7.7	4.8	6.6	2.3
9	6.0	10.7	7.1	11.3	9.0	7.5	9.8	6.6	6.2	5.7
\bar{X}	8.78	8.97	8.95	8.73	9.12	10.80	9.35	7.95	9.70	8.68

ceiving the vitamin A supplement tended to be lower ($P < 0.05$) than of the calves from basal dams. However, the vitamin A levels in the plasma of calves from dams receiving the basal ration plus vitamin A were significantly higher

($P < 0.001$) than those of the calves from dams receiving the basal ration alone. The plasma vitamin A was significantly higher ($P < 0.01$) on each individual day except at 2 and 10 days of age in the calves from dams receiving vitamin A.

The carotene and vitamin A storage in the liver at 10 days of age is shown in table 2. The liver storage of carotene and vitamin A in calves from dams fed

TABLE 2

The effect of prepartum supplementation of vitamin A on the liver storage of carotene and vitamin A at 10 days of age in calves fed reconstituted skim milk

Calf no.	Carotene		Vitamin A	
	$\gamma/\text{g.}$	Total γ	$\gamma/\text{g.}$	Total γ
<i>Basal dams</i>				
1	0.24	148	0.29	178
2	0.50	361	0.21	152
3	0.71	432	0.15	91
\bar{X}	0.483	313.7	0.217	140.3
<i>Basal + vitamin A dams</i>				
4	0.20	114	1.28	727
5	0.14	83	1.00	593
6				
7	0.16	98	7.80	4789
8	0.44	314	2.45	1747
9	0.55	459	5.19	4334
\bar{X}	0.298	213.6	3.544	2438.0

supplementary vitamin A paralleled the blood plasma levels; that is, the liver storage of carotene was reduced and that of vitamin A was increased as compared with calves from dams on the basal ration alone. The difference in vitamin A was statistically significant ($P < 0.005$), but the difference in carotene was not.

DISCUSSION

These data indicate that the vitamin A stored by the fetus can be utilized by the young calf and should be considered in the nutrition of the young calf in addition to colostrum.

Previous work (4, 17) has shown that the feeding of supplementary vitamin A during the prepartum period has raised the levels of vitamin A in the plasma during the neonatal period in both calves and lambs. One source of this vitamin A is believed to be fetal storage. This emphasizes the importance in calf studies of evaluating critically the nutritional history of the dam.

Other workers have reported the apparent depression of the carotene level when supplementary vitamin A is fed. Wise *et al.* (19) recently have reviewed this decrease in respect to the lactating cow, and other workers (3, 11) have reported a similar finding when vitamin A is fed directly to the calf. This experiment corroborates the finding of Esh *et al.* (5) that intrauterine nutritional influences seemed to depress the plasma carotene.

The possibility that reconstituted skim milk may stimulate the mobilization of storage depots of vitamin A should not be overlooked. Several reports (3, 7) of such an effect can be found in the literature. It would seem desirable, therefore, to eliminate this factor by feeding a whole milk with a uniform, relatively low content of vitamin A. Preliminary work (13) has indicated that fetal storage of vitamin A contributes to the higher blood plasma levels in young calves, especially after 2 weeks of age, when a standardized colostrum and whole milk is fed.

SUMMARY

One million U.S.P. units of vitamin A were fed daily to the dam for 30 days prior to the calculated parturition date and the effect of this supplement on blood plasma levels and liver storage of carotene and vitamin A was measured in six young male dairy calves fed only reconstituted skim milk. Parallel measurements were made on three control calves. These data indicate that the fetal storage of vitamin A can be utilized by the newborn calf. This is shown by the decrease in blood plasma levels of carotene, the increase in blood plasma levels of vitamin A, and the greater liver storage of vitamin A at 10 days of age of calves from dams fed supplementary vitamin A.

ACKNOWLEDGMENTS

The authors are most grateful to F. Warren and G. Farrington for the care of the experimental animals and to Miss M. W. Dicks and R. J. Slate for technical assistance during the course of the experiment. Further acknowledgment is due C. I. Bliss, Storrs Agricultural Experiment Station Biometrician, for considerable aid in the statistical analysis of the data and in the preparation of the paper, and to Mrs. L. Griswold, Department of Poultry Husbandry, for the carotene analysis of the feedstuffs.

REFERENCES

- (1) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. *Ind. Eng. Chem., Anal. Ed.*, **10**: 101-102. 1944.
- (2) DAVIES, A. W. The Colorimetric Determination of Vitamin A by Alkali Digestion. *Biochem. J.*, **27**: 1770-1775. 1933.
- (3) EATON, H. D., SPIELMAN, A. A., LOOSLI, J. K., THOMAS, J. W., NORTON, C. L., AND TURK, K. L. The Effect of a Massive Dose at Birth of Vitamins A and D upon Blood Plasma Levels and Liver Storage in Holstein Calves. *J. Dairy Sci.*, **30**: 795-802. 1947.
- (4) EATON, H. D., CHRISTIAN, J. A., DAUGHERTY, F. C., SPIELMAN, A. A., AND MATTERSON, L. D. The Effect of Crude Soybean Lecithin on the Absorption and Utilization of Vitamin A Fed Prepartum to the Ewe and Sow. *J. Animal Sci.*, **8**: 224-233. 1949.
- (5) ESH, G. C., SUTTON, T. S., HIBBS, J. W., AND KRAUSS, W. E. The Effect of Soyaphosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, **31**: 461-478. 1948.
- (6) FOUNTAINE, F. C., PARRISH, D. B., ATKESON, F. W., AND HUGHES, J. S. The Effect of a Ration Limited to Pasture During the Dry-rest period on the Vitamin A and Carotenoid Content of the Blood of the Newborn Calf (Abstract). *J. Animal Sci.*, **7**: 525. 1948.

- (7) JACOBSON, W. C., AND THOMAS, J. W. Some Irregular Fluctuations in the Vitamin A Level of Blood Plasma Produced by Ration Changes in Calves (Abstract). *J. Dairy Sci.*, 31: 671-672. 1948.
- (8) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, 24: 1055-1065. 1939.
- (9) MOORE, L. A., AND ELY, R. Extraction of Carotene from Plant Material. A Rapid Quantitative Method. *Ind. Eng. Chem., Anal. Ed.*, 13: 600-601. 1941.
- (10) NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING.—(University of Reading, England). Annual Rept. for the year ended 30th September, 1947.
- (11) NELSON, D. H., AND CUNNINGHAM, O. C. The Effect of a Vitamin A Supplement in Raising Calves with a Starter Containing No Animal Protein. *New Mexico Agr. Expt. Sta. Bull.* 333. 1946.
- (12) NELSON, W. L., LOOSLI, J. K., LOFGREN, G., AND YAGER, N. Carotene Retention in Alfalfa Meal. Effect of Blanching, Packaging, and Storage Temperature. *Ind. Eng. Chem.*, 40: 2196-2198. 1948.
- (13) NEZVESKY, L., SPIELMAN, A. A., EATON, H. D., JOHNSON, R. E., AND MATTERSON, L. D. Unpublished data. Animal Industries Dept., University of Connecticut. 1949.
- (14) PHILLIPS, P. H. The Question of Supplementary Vitamins for Calves. *J. Am. Vet. Med. Assoc.*, 111: 218-220. 1947.
- (15) SNEDECOR, G. W. *Statistical Methods*. 4th ed. Iowa State College Press, Ames. 1946.
- (16) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. The Placental Transmission and Fetal Storage of Vitamin A and Carotene in the Bovine. *J. Dairy Sci.*, 29: 707-715. 1946.
- (17) SPIELMAN, A. A., EATON, H. D., LOOSLI, J. K., AND TURK, K. L. The Effect of Prepartum Vitamin A Supplementation on the Health and Performance of the Young Calf. *J. Dairy Sci.*, 32, 4: 367-374. 1949.
- (18) WISE, G. H., CALDWELL, M. T., AND HUGHES, J. S. The Effect of the Prepartum Diet of the Cow on the Vitamin A Reserves of Her Newborn Offspring. *Science*, 103: 616-618. 1946.
- (19) WISE, G. H., ATKESON, F. W., CALDWELL, M. J., PARRISH, D. B., AND HUGHES, J. S. Effect of High Vitamin A Intake on Milk and Fat Yields and on Vitamin A Constituents in Milk, Blood, and Livers of Dairy Cows. *J. Dairy Sci.*, 30: 279-291. 1947.

THE EFFECT OF ADDED AMINO ACIDS ON THE FLAVOR OF CHEDDAR CHEESE MADE FROM PASTEURIZED MILK¹

R. J. BAKER AND F. E. NELSON

Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

The protein in cheese curd has been shown to undergo a series of chemical changes during the ripening process, until amino acids and ammonia are present in some quantities, in addition to the more complicated degradation products. Whether certain protein decomposition products, materials produced by action upon butterfat or products resulting from changes in other components of the cheese curd are responsible for the characteristic Cheddar flavor has not been established satisfactorily, partly because of the complexity of the system in which the changes are occurring.

Davies *et al.* (2) added, at the time of salting, various chemical compounds which might affect the growth and metabolism of bacteria or the activity of rennet enzymes. Cystine was added in concentrations of 0.01 and 0.02 per cent, but it had no significant effect on the rate of ripening, even though the flavor score of the cheese made with cystine was slightly higher than that of the control cheese. No differences were noted in the amounts of the various nitrogen fractions. After the present investigation had been completed, Harper and Swanson (4) indicated that mixtures of amino acids may contribute to the flavor of Cheddar cheese.

The purpose of the present study was to test the possibility that the addition of various amino acids to cheese curd made from pasteurized milk might contribute to production of the typical flavor and aroma characteristic of a fine raw-milk Cheddar cheese ripened properly, either directly or as substrata upon which microorganisms or enzymes might act.

METHODS

Manufacture of Cheddar cheese. The milk used in all cheese was mixed herd milk with a fat content varying from 3.1 to 3.8 per cent. It was pasteurized at 143° F. for 30 minutes in a spray-vat pasteurizer. For lots 1 through 8, 170 lb. of milk were used and for lots 9 through 12, 125 lb. of milk were used. In all lots the method of manufacture was essentially that of Wilson (7), with the modification that after the curd was salted, it was weighed into separate 2.5-lb. quantities. Lots 1 through 8 required six separate quantities from each quantity of curd, while lots 9 through 12 required five quantities. The amino acid-salt mixture described later then was incorporated into the 2.5-lb. quantities of curd and allowed to dissolve for approximately 10 minutes before the curd was placed in small wooden hoops. The hoops were used to produce a cheese 5 inches in diameter and 2.5 inches thick, weighing approximately 2.5 lb. The pressure was applied slowly in the press to minimize the amount of whey expressed from the cheese until the

Received for publication April 1, 1949.

¹ Journal paper no. J1628 of the Iowa Agricultural Experiment Station, Ames. Project no. 652.

amino acids had been in contact with the curd for some time. The cheeses were ripened at 60° F.

After curing for 3 days, samples of cheese were obtained and determinations were made of the fat, moisture and total chlorides. The various cheeses were sufficiently similar in composition, both within a lot made at one time with different amino acids and between the various lots within a series, that differences in flavor could not be attributed to variations in gross composition. Therefore, the data on composition are not presented.

Organoleptic examination. Lots 1 through 8 were examined every 4 weeks for a period of 24 weeks. Lots 9 through 12 were examined at 2-week intervals for a period of 12 weeks, and again at the age of 24 weeks. The cheeses in each lot were compared with the control to determine any difference in flavor characteristics, as the emphasis was upon possible increase in characteristic Cheddar flavor, rather than on a score based upon commercial scoring practices. Each lot was ranked from 1 to 6 or 1 to 5, as determined by the number of cheeses in the lot, with 1 being the most desirable cheese and 6 or 5 being the least desirable. In case no differences were noted between certain cheeses in a lot, an average of the rankings covered by the undifferentiated cheeses was assigned to each of the cheeses thus grouped.

Preparation of amino acids. For this study DL-alanine, L-arginine, DL-aspartic acid, L-cystine, DL-glutamic acid, glycine, L-histidine, L-hydroxyproline, DL-isoleucine, DL-leucine, L-lysine, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, L-tryptophan, L-tyrosine and DL-valine were used. In lots 1 through 8, 0.5 g. (0.04 per cent of the weight of the cheese curd) of the pure amino acid was weighed out and combined with 5 g. of cheese salt, the resulting mixture being added to 2.5 lb. of curd. This mixture was ground thoroughly in a mortar and pestle, to obtain a finely divided material. The amino acids chosen for lots 9 through 12 were those which had given what was apparently the most desirable cheese in previous trials and included glycine, methionine, tyrosine, serine, glutamic acid, arginine, aspartic acid and valine. These eight amino acids were added to separate cheese in amounts of 0.5 g. (0.04 per cent) and 1.5 g. (0.13 per cent). In each case the amino acid was ground with 5 g. of salt.

The amount of amino acid originally chosen, 0.5 g., was based on the assumption that it was a large enough amount to produce any probable beneficial effects, as it would represent the freeing of a considerable fraction of the acid in question from the proteins. In lots 9 through 12, the use of 1.5 g. of each amino acid chosen was to determine if the larger amount would have any effect upon the flavor produced or upon the rate of bacterial growth.

Microbiological examination. Microbiological examination of the cheese in lots 9 through 12 was thought advisable to determine any correlation between the bacterial counts and the flavor of the cheese, or any effect of the amino acids upon bacterial development. The sampling and plating procedures were those proposed by the American Public Health Association (1). Tomato juice agar (5) was the plating medium, and the plates were incubated at room temperature for 5 days. Typical colonies were picked from the plates and examined for morphological

TABLE 1

Ranks according to flavor within lots of cheese in which 0.5 g. of amino acid had been incorporated at salting

Lot	Amino acids used	Rank at						Total rank
		4 wk.	8 wk.	12 wk.	16 wk.	20 wk.	24 wk.	
First series								
1	Control	6	6	6	4.5	6	6	34.5
	Valine	4	4.5	4	4.5	4	4	25
	Leucine	3	4.5	5	4.5	5	5	27
	Glutamic acid	2	2	2	1	3	3	13
	Serine	1	1	2	2	1	1	8
	Arginine	5	3	2	4.5	2	2	18.5
2	Control	5	3	5	1	2	2	18
	Aspartic acid	3	2	3.5	3	2	2	15.5
	Proline	1	5	1	5	4	4	20
	Phenylalanine	2	4	3.5	3	5	5	22.5
	Tyrosine	4	1	2	3	2	2	14
	Histidine	6	6	6	6	6	6	36
3	Control	3.5	5	5	5	5	4	27.5
	Threonine	3.5	3.5	3	4	4	4	22
	Methionine	3.5	1.5	1.5	2	2	1	11.5
	Lysine	3.5	6	1.5	2	2	4	19
	Tryptophan	3.5	3.5	4	6	6	4	27
	Isoleucine	3.5	1.5	6	2	2	4	19
4	Control	3	4	6	5	6	3.5	27.5
	Alanine	6	4	3.5	2	4	3.5	23
	Glycine	3	4	1	1	1	1	11
	Hydroxyproline	3	6	3.5	6	5	3.5	27
	Cystine	5	2	3.5	3	2	6	21.5
Second series								
5	Control	4	5	6	4	4.5	3.5	27
	Valine	2	2	2	1	2	1	10
	Aspartic acid	3	2	1	3	4.5	3.5	17
	Threonine	1	2	3	6	4.5	3.5	20
	Alanine	5	4	5	5	4.5	6	29.5
6	Control	4	5	6	6	4.5	4.5	30
	Leucine	5	1	4	5	4.5	4.5	24
	Proline	1	6	5	3	4.5	4.5	24
	Methionine	2	3.5	1	2	2	2	12.5
	Glycine	3	3.5	2	1	1	1	11.5
	Isoleucine	6	2	3	4	4.5	4.5	24
7	Control	5	5	5	5	4	4	28
	Cystine	1	3	3	2.5	2	2	13.5
	Tyrosine	3	2	1	2.5	2	2	12.5
	Glutamic acid	3	1	2	2.5	2	2	12.5
	Histidine	6	6	6	6	6	6	36
	Lysine	3	4	4	2.5	5	5	23.5
8	Control	4	2	4	5	6	5	26
	Arginine	4	6	3	1.5	1	2	17.5
	Phenylalanine	4	5	6	6	5	6	32
	Hydroxyproline	1	4	5	4	4	3	21
	Tryptophan	4	3	2	3	3	4	19
	Serine	4	1	1	1.5	2	1	10.5

characteristics. For the direct microscopic count, a 0.01 ml. sample was pipetted directly from the 1:10 dilution, spread in a film over an area of 1 cm.², dried, treated with xylol and stained with the Newman-Lampert stain.

RESULTS

The data from the first two series of cheese, in which 0.5 g. of amino acid was added to 2.5 lb. of curd at the time of salting, are presented in table 1. The only difference between the two series is the association within lots within the series. Since the differences in flavor between the cheeses in one lot commonly were small, the error of ranking undoubtedly is considerable.

Histidine consistently contributed a very objectionable and characteristic flavor and was placed last in each comparison. An aqueous solution of histidine had this same characteristic flavor, indicating that the amino acid as such imparted the undesirable flavor to the cheese. Cheese to which other amino acids had been added showed a slight tendency in some cases or no tendency in other cases toward improvement over the controls. On the basis of these results, eight amino acids which showed some possibility of affecting the flavor favorably were chosen for further trials in a third series.

TABLE 2

Ranks according to flavor within lots of cheese in which 0.5 g. and 1.5 g. amino acid had been incorporated at salting

Lot	Amino acids used	Amt.	Rank at							Total rank
			2 wk.	4 wk.	6 wk.	8 wk.	10 wk.	12 wk.	24 wk.	
Third series										
(g.)										
9	Glycine	0.5	3.5	2	3	3	3	3.5	3	21
	Glycine	1.5	5	2	3	3	3	3.5	3	22.5
	Control		3.5	2	3	3	3	3.5	3	21
	Methionine	0.5	1	4.5	3	3	3	3.5	3	21
	Methionine	1.5	2	4.5	3	3	3	1	3	19.5
10	Tyrosine	0.5	1.5	1	3	3	3.5	5	3	20
	Tyrosine	1.5	5	3.5	3	3	3.5	3	3	24
	Control		3.5	3.5	3	3	5	3	3	24
	Serine	0.5	3.5	3.5	3	3	2	3	3	21
	Serine	1.5	1.5	3.5	3	3	1	1	3	16
11	Glutamic acid .. .	0.5	2.5	3	3	3	5	3	3	22.5
	Glutamic acid .. .	1.5	5	3	3	3	3	3	3	23
	Control		2.5	3	3	3	3	3	3	20.5
	Arginine	0.5	2.5	3	3	3	3	3	3	20.5
	Arginine	1.5	2.5	3	3	3	1	3	3	18.5
12	Aspartic acid .. .	0.5	4.5	3	3	3	1	3	2.5	20
	Aspartic acid .. .	1.5	2	3	3	3	4.5	3	5	23.5
	Control		2	3	3	3	4.5	3	2.5	21
	Valine	0.5	2	3	3	3	2.5	3	2.5	19
	Valine	1.5	4.5	3	3	3	2.5	3	2.5	21.5

The data on the rankings of the third series of cheese are presented in table 2. This series consisted of cheese made with 0.5 g. and 1.5 g. amino acid per 2.5 lb.

of curd. There was no appreciable difference in the level of flavor development, as indicated by the total ranks for the 24-week curing period. Serine added at the rate of 1.5 g. per 2.5 lb. of curd showed a possibility of some desirable effect at the 12-week ranking, but no difference was noted at the 24-week ranking. Data for the other additions were too inconsistent to indicate any potential desirable effect of these compounds upon flavor production in Cheddar cheese.

Little effect of the added amino acids upon the microbial population could be demonstrated. Generally the types varied as would be anticipated for normal cheese, with the greatest number of lactic acid streptococci present early in the curing period, followed by a brief period when yeasts were evident and this in turn usually followed by an increase in the numbers of gram-positive non-sporulating, long and short rods. The gram-positive rods varied from a negligible number in the case of the use of 1.5 g. of methionine to the predominant type during the latter part of the ripening period of those cheese made with added serine, but the results seemed to be conditioned more by the lot of milk used than by the added amino acid.

DISCUSSION

The addition of individual amino acids to cheese curd made from pasteurized milk appeared to have little consistent effect upon the flavor of the resultant Cheddar cheese. One exception is that the addition of histidine resulted in a very definitely undesirable flavor defect, and another exception is the possible slight beneficial influence of serine. Other amino acids either gave no improvement or results which were so inconsistent as to prevent the drawing of definite conclusions that the flavor was improved as a result of their addition. These results are in accord with previous reports, such as those of Van Slyke *et al.* (6) and Freeman and Dahle (3), that additional proteolysis, particularly that resulting from the use of quantities of rennet greater than normally employed, did not result in additional or accelerated flavor development. However, these earlier studies did not determine definitely that breakdown to amino acids was involved.

Serine possibly increased the numbers of lactobacilli present in the cheese; this is of interest because the cheese to which 1.5 g. of serine had been added was the only one of series 3 which showed possibly significant evidence of improved flavor. The relatively small numbers of gram-positive rods in other lots of the third series of cheese, both among the control cheese and those cheese to which amino acids were added may have been a factor in the lack of flavor development.

Although a known amount of each amino acid was added to the curd, no studies of the amounts of amino acids retained in the cheese were made, and the data should be interpreted with that limitation in mind. The varying solubilities of different amino acids may have affected the amount of each which was retained in the cheese, although the lots in which increased amounts were used without difference in effect would indicate that variations in retention are not an important factor.

Another factor which should be considered is that, although the addition of most amino acids to Cheddar cheese made from pasteurized milk failed to produce consistent improvement in flavor, the use of amino acids in cheese made from raw milk might produce different results because of the different bacteria and

enzyme systems which presumably are factors in the ripening of such cheese. Possibly some of the inconsistencies noted in the present study could be attributed to differences in the bacterial flora of the various lots of cheese. The testing of some of the potentially favorable amino acids by addition to cheese containing selected strains of bacteria might offer some interesting possibilities.

The preliminary report of Harper and Swanson (4), which appeared after the present study had been completed, indicates that amino acid mixtures may have a greater favorable influence on flavor development in Cheddar cheese than do the various amino acids added singly. This is an aspect of the problem which was not explored in the present investigations.

SUMMARY AND CONCLUSIONS

Nineteen of the amino acids normally present in casein were added in 0.5 g. quantities to 2.5 lb. of curd made from pasteurized milk, as a possible means of improving the flavor of the resulting cheese, either directly or as substrata for enzyme or microbial activity.

Histidine contributed a definitely undesirable flavor. Addition of the other amino acids resulted in cheeses indistinguishable from the controls in most cases and slightly but inconsistently better in a few cases. The eight amino acids which the first two series indicated might possibly contribute something to the flavor were used in 0.5 g. and 1.5 g. amounts per 2.5 lb. of curd in a third series of cheese to determine any effect of the increased amino acid content upon the typical flavor of Cheddar cheese. These eight amino acids had little or no effect on the flavor ranking and bacterial development except that serine possibly had a favorable effect on both flavor and bacteria.

The addition of amino acids to Cheddar cheese made from pasteurized milk had no consistent desirable effects upon flavor development under the conditions of this study.

The assistance of F. J. Babel in judging a number of the lots of cheese is acknowledged.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Microbiological Methods for the Examination of Cheese (tentative). Mimeograph, American Public Health Association, New York, N. Y. 1944.
- (2) DAVIES, W. L., DAVIS, J. G., DEARDEN, D. V., AND MATTICK, A. T. R. Studies in Cheddar cheese. V. The Effect of Chemical Substances on the Ripening Process. *J. Dairy Research*, 8: 92-104. 1937.
- (3) FREEMAN, T. R. AND DAHLE, C. D. Rate of Ripening in Cheddar Cheese. *Penn. Agr. Expt. Sta. Bull.* 362. 1938.
- (4) HARPER, W. J., AND SWANSON, A. M. Studies of Amino Acids in Cheddar Cheese during Ripening (Abstract). *J. Dairy Sci.*, 31: 715-716. 1948.
- (5) LANE, C. B., AND HAMMER, B. W. Bacteriology of Cheese. II. Effect of *Lactobacillus casei* on the Nitrogenous Decomposition and Flavor Development in Cheddar Cheese Made from Pasteurized Milk. *Iowa Agr. Expt. Sta. Research Bull.* 190. 1935.
- (6) VAN SLYKE, L. L., HARDING, H. A., AND HART, E. B. Rennet-enzyme as a Factor in Cheese Ripening. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 233. 1903.
- (7) WILSON, H. L. Manufacture of American Cheese from Pasteurized Milk. *Natl. Butter Cheese J.*, 33, 2: 18, 20, 22, 24-28. 1942.

THE PHYSICAL PROPERTIES OF EVAPORATED MILK WITH RESPECT TO SURFACE TENSION, GRAIN FORMATION AND COLOR

VICTOR NELSON

*Central Research Department, Food Machinery and Chemical Corporation,
San Jose, California*

The sterilization processes used in the manufacture of evaporated milk are designed not only for sterility but for those physical characteristics which improve the quality of the milk. As a rule, most manufacturers of evaporated milk grade the sterilized milk in terms of viscosity, grain and color; the relative importance assigned to these properties varies, depending on the point of view of the manufacturer.

In the quest for improved sterilization processes, the lack of organization of processing data has been an impediment to a comprehensive understanding of the sterilization process. A knowledge of the relationship existing between the color, viscosity, grain formation and surface tension for processes in general would assist greatly in predicting the effects of contemplated changes in processing.

Considerable attention has been given to the problems arising in the sterilization of milk. Bell *et al.* (1) found that high temperature-short time sterilized evaporated milk thickened on storage, but if the milk was filled into cans after the high temperature-short time sterilization process and the color increased by further heating, the gelation tendency was reduced greatly, if not eliminated. Holm *et al.* (3) determined the relationship between the cooking time and the temperature necessary for coagulation. Later, the logarithmic nature of the heat coagulation was noted by Webb and Holm (5) in test tube experiments. While many of the phenomena associated with the processing of evaporated milk are recorded in the literature, little if any attention has been given to the character of the heat coagulation or its relationship to color, viscosity and surface tension.

The purpose of this paper is to examine the results obtained in various sterilization processes with reference to grain formation, surface tension, viscosity and color and to show the relationship of these measurements to one another.

METHODS AND APPARATUS

The apparatus consisted of a thermostatically controlled oil bath, 75 mm. \times 10 mm. test tubes, a wire tray for holding the tubes, a cold water bath for cooling the tubes quickly after heating, a Beckman spectrophotometer for reflectance measurements, a Du Noüy tensiometer, a Mojonnier-Doolittle Universal viscosimeter, a pilot sterilizer and a Brown recording potentiometer.

The method adopted for the analysis of sterilization processes is based on a comparison of the effects of time and temperature on standard evaporated milk in test tube experiments with the effects of time and temperature on similar milk processed in commercial or like equipment.

Received for publication April 4, 1949.

EXPERIMENTAL PROCEDURE

A 50-lb. lot of raw milk was heated in a jacketed hot well to 203° F. and held at this temperature for 10 minutes. The time required for the milk to reach 203° F. was 10 to 12 minutes. Following this hot well treatment, the milk was drawn into a pilot evaporator where evaporation of water from the milk was conducted under vacuum at a temperature of 125° F. The average time for the evaporation of a 50-lb. lot of milk was 15 minutes. After evaporation, the milk was heated to 140° F. and homogenized at 2,500 lb. per square inch. Since more water was evaporated from the milk than is required for standard milk, it was standardized to 7.9 per cent fat and 26.5 per cent total solids by the addition of water. Finally, 2 ml. of this milk were inserted carefully into the small test tubes with a hypodermic needle. The tubes were sealed over a small flame, the hot tip of each tube being drawn into a loop so that the tubes could be suspended on a wire and placed in a tray.

Four lots of samples, each consisting of ten prepared tubes of milk at room temperature, were processed in the oil bath at the temperatures given in table 1. After processing, tubes were withdrawn at predetermined intervals, cooled in the water bath, dried, numbered and later the contents were analyzed for color and surface tension.

The relative color change was recorded in terms of the reflective index as used by Nelson (4). Surface tension determinations were made on the Du Noüy tensiometer after standardizing the instrument with weights, but the value in dynes per centimeter is relative, since the minor adjustments and corrections were not observed in the manipulation of the instrument. The essential part of these data is given in table 1.

TABLE 1

The effect of time and temperature on the color and surface tension of evaporated milk heated in small sealed test tubes

233.5° F.				244.5° F			
Sample	Time	Reflectance 520 m μ	Surface tension ^b	Sample	Time	Reflectance 520 m μ	Surface tension
	(min.)	(%)	(dynes/cm.)		(min.)	(%)	(dynes/cm.)
1	40	55.3	54.7	1	28	50.0	54.1
2	45	52.8	56.9	*2	31	47.8	56.0
*3	50	50.0	57.6	3	34	45.5	56.0
4	55	47.5	57.7	4	37	43.5	56.0
250.5° F.				257° F.			
1	20	50.3	54.5	1	13	54.9	52.3
*2	22	47.3	56.0	*2	16	48.8	55.4
3	24	44.5	56.0	3	18	45.8	55.4
4	26	42.0	56.9	4	20	43.3	55.8

* Curve 1, fig. 1 constructed from these data after correction for temperature lag.

^b Surface tension of unsterilized milk was 50.3 dynes/cm. at 75° F. Reflectance of unsterilized evaporated milk at 520 m μ was 80.3%.

Preparation of a surface tension curve. A preliminary curve representing points of equivalent surface tensions with respect to temperature and time was

drawn from the data given in table 1. For greater accuracy, it was thought desirable to evaluate the time required for the tubes of milk to approach within 1° F. of the bath temperature. The method used was essentially that of Bigelow *et al.* (2). Thermocouples were inserted into milk at 75° F. contained in the small tubes described previously and these tubes were immersed in a constant temperature oil bath maintained at 200° F. The average time-temperature data obtained plotted as a straight line on semi-log paper and it was assumed that this relationship continued within the limits of the processing temperatures studied. The slope of this line was expressed as the number of minutes required to cross one log cycle, in this case 1.8 minutes. This slope was used for the four processes studied.

The time calculated for the milk in the tubes to reach the desired bath temperature varied from 3 minutes to attain the bath temperature of 233° F. to 3.5 minutes to attain the bath temperature of 256° F. This lag time was almost entirely without effect on the milk, since approximately 2.5 to 3.0 minutes were required to attain 200° F. and temperatures below 200° F. have relatively little effect on milk when compared with temperatures above 200° F. Because the time of processing was relatively long, there was little object in converting the 0.5 minute period above 200° F. into effective time at bath temperature. Therefore, a lag time of approximately 3 minutes was subtracted from the observed time given in table 1 and the preliminary curve based on the observed time of table 1 was replaced by curve 1, figure 1. This latter curve has a somewhat greater slope than the preliminary curve.

It was observed that the surface tension of evaporated milk, taken after heat processing, increased generally as the apparent viscosity increased. This increase in surface tension is in conformity with the well-known effect of proteins on the surface tension of a liquid; that is, a reduction in protein activity at a surface increases the surface tension. Therefore, in the preparation of a curve representing surface tension at the temperatures and time of processing given, for example, in table 1, the surface tension provides an indirect means of estimating the probable true extent of heat coagulation with greater precision than by judging from the amount of grain formation.

It was not found convenient to control the room temperature at the time of analysis; therefore, the surface tension values chosen for equivalence were taken at the time of abrupt change in the rate of rise, which generally was near the maximum value attainable. In general, most of the rise in surface tension occurred during the final third of the processing period. This period of increasing surface tension was marked by an initial rapid rise, then a period of little change and finally by a small further rise to a maximum value.

Preparation of evaporated milk samples. The pilot sterilizer used in obtaining the data on coagulation and color of milk sterilized in cans was designed so that the processed milk obtained would be comparable to evaporated milk obtained from a commercial Anderson-Barngrover sterilizer when similar processes were used. The pilot sterilizer used is a single-unit, cylindrical cooker, approximately 20 inches in length and 30 inches in diameter, in which a nine pocket reel is installed. An entrance valve is located on top, so that cans may be entered in a

manner similar to that in a commercial A-B sterilizer. A flexible metal belt is installed under the reel so that the cans may be given additional rolling to approximate more closely the conditions in the A-B sterilizer. Removal of a plate on the side of the sterilizer gave access to the cans after processing.

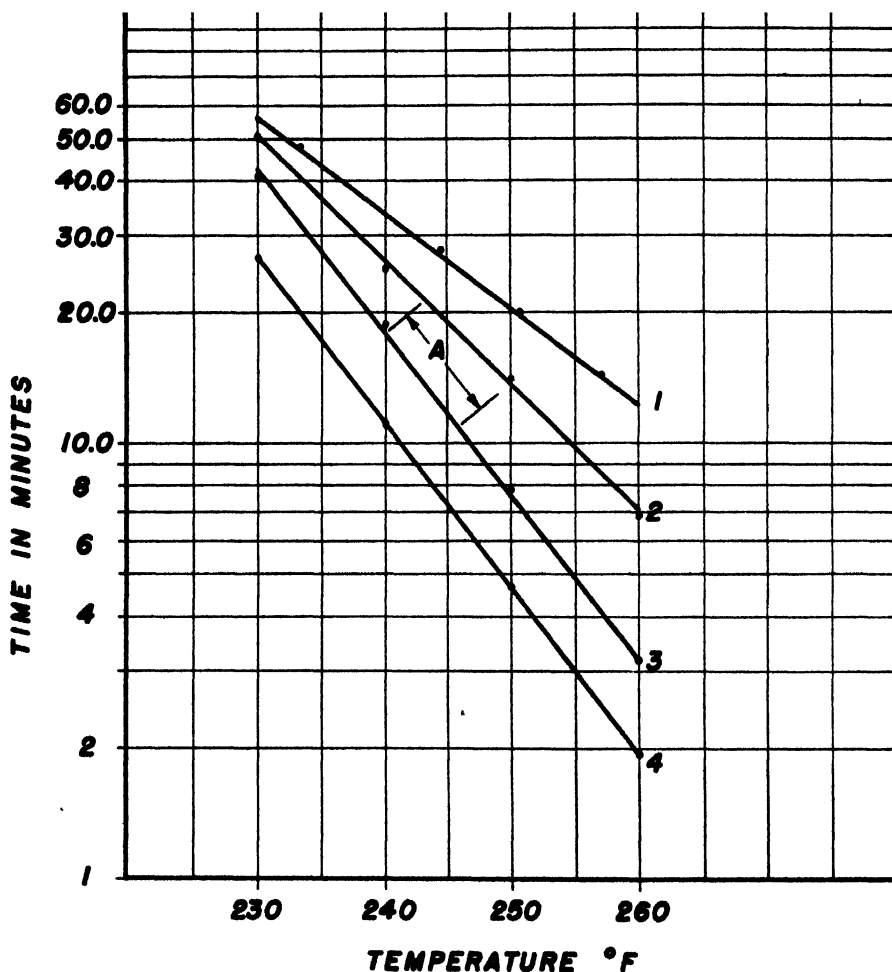


FIG. 1. The relationship between time and temperature of heating as it affects grain, surface tension and color of heat processed evaporated milk.

Curve 1. Represents points of equal color and surface tension as determined in test tube trials. Curves 2, 3 and 4 represent equivalent grain points in processed evaporated milk for raw milk forewarmed as follows: For 2-milk forewarmed to 210° F., held 10 minutes, followed by heating to 250° F. for 10 seconds; for 3-milk forewarmed to 210° F. and held 10 minutes; for 4-milk forewarmed to 195° F. without holding time. Commercial sterilization processes are indicated by Zone A.

Samples of standard evaporated milk in 14.5 oz. commercial milk cans were entered through a valve into the cooker maintained at 227° F. The time of pretreatment was 9 minutes. At the expiration of the pretreatment period, the temperature of the cooker was raised quickly (10–15 seconds) to the desired temperature and held at this temperature until the desired degree of grain formation was obtained. At the end of this processing period, the milk was cooled quickly by the addition of cold water to the cooker. Continuous rotation of the reel was maintained throughout the process. Grain formation in the milk was judged by the amount of coagulation noted in a film of milk formed by means of a wire loop or a similar method. The degree of graining was noted, in order of increasing severity, as very slight film, slight film, film and heavy film. Grain formation seldom was developed to the point of visibility on the back of a spoon. Data typical of these pilot runs are recorded in table 2. Curves 2, 3 and 4 of figure 1 were derived from these data.

TABLE 2
The effect of heat on the physical properties of evaporated milk

Group	Sample	Viscosity Mojonnier (75° F.)	% Re- flectance 520 mμ (%)	Grain	Surface tension (dynes/cm.)	Cooker ^a	
						Time (min.)	Temp. (° F.)
2 ^b	1a	30	62.8	sl. film	51.4	7.0	260
	2a	45	59.8	sl. film	54.2	13.5	250
	3a	67	57.5	film	55.7	23.5	240
	4a	125	54.8	hy. film	58.1	47.5	230
3 ^c	1b	30	73.6	sl. film	53.2	3.5	260
	2b	40	70.3	film	54.1	7.0	250
	3b	51	65.3	film	54.3	17.0	240
	4b	57	60.0	film	55.0	37.0	230
4 ^d	1c	30	81.2	sl. film	50.4	2.0	260
	2c	45	78.8	sl. film	51.8	4.0	250
	3c	48	75.5	sl. film	53.2	10.0	240
	4c	60	70.5	film	56.2	24.0	230

^a Cooker time preceded by 9-min. process at 227° F.

^b Forewarning process for group 2 was 210° F. for 10 min. followed by 10 sec. at 250° F.

^c Group 3 was forewarmed to 210° F. and held 10 min.

^d Group 4 was forewarmed to 195° F. but not held.

Conversion of process time into time at a given temperature. The evaluation process used herein is similar to that used by Bigelow *et al.* (2) for the conversion of the effect of time at one temperature into the effect of time at another temperature. The conversion is illustrated in figures 2 and 3. For example, figure 2 illustrates the heat penetration curve of a can of milk from the end of the pretreatment process at 227° F. through the 7-minute process at 250° F. and for 1.5 minutes of the cooling period. The slope of the heat penetration curve is 2.9 minutes per log cycle; this slope is used for all processes and was determined in this laboratory. The log cycle for the temperature range 249 to 250° F. is omitted for convenience in plotting and on account of the negligible effect on the calculated process time.

From the data of figure 2 a second curve is plotted (figure 3) in which the process time of figure 2 is plotted as the abscissa and the reciprocals of the time on curve 1, figure 1 corresponding to the process temperatures shown on figure 2 are plotted as ordinates. For example, after 2 minutes in the cooker the temperature of the milk (center of can) is 240° F. as shown on figure 2. The time for this temperature on curve 1 figure 1 is 34.5 minutes. The reciprocal of this time is

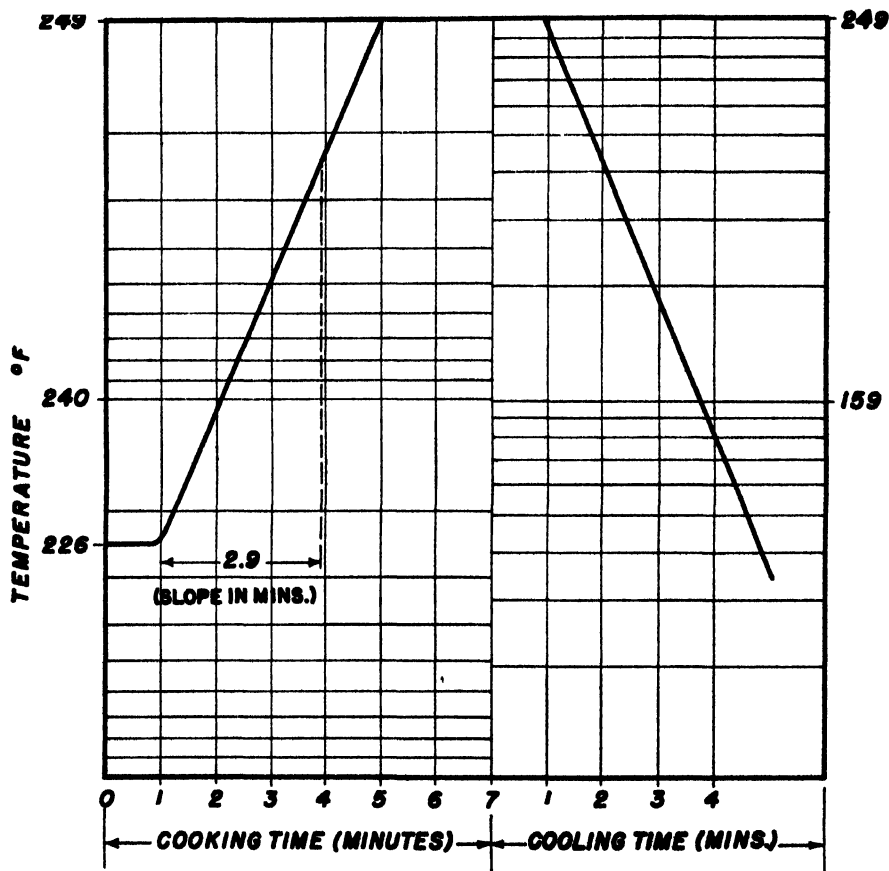


Fig. 2. Rate of heating and cooling of evaporated milk during processing.

plotted on figure 3 as ordinate of abscissa 2. The area under this curve (solid line) represents the part of the 8.5 minutes that is effective at 250° F., in this case 6.8 minutes. The pretreatment time (not shown but derived by similar methods) is equivalent to 1.2 minutes at 250° F. The total time plotted is 8 minutes as shown on curve 3, figure 1. The data used in the illustration are derived from the data for sample 2b, table 2.

Table 2 is a compilation of data for samples of evaporated milk heated as indicated to locate points of grain equivalence. Because the limited time and number

of samples available for this experiment were insufficient to permit determination of the rate of surface tension rise and grain formation, a second lot of evaporated milk was prepared for this purpose. In this second experiment the raw milk was forewarmed by the batch method to 205° F. and held 5 minutes at this tempera-

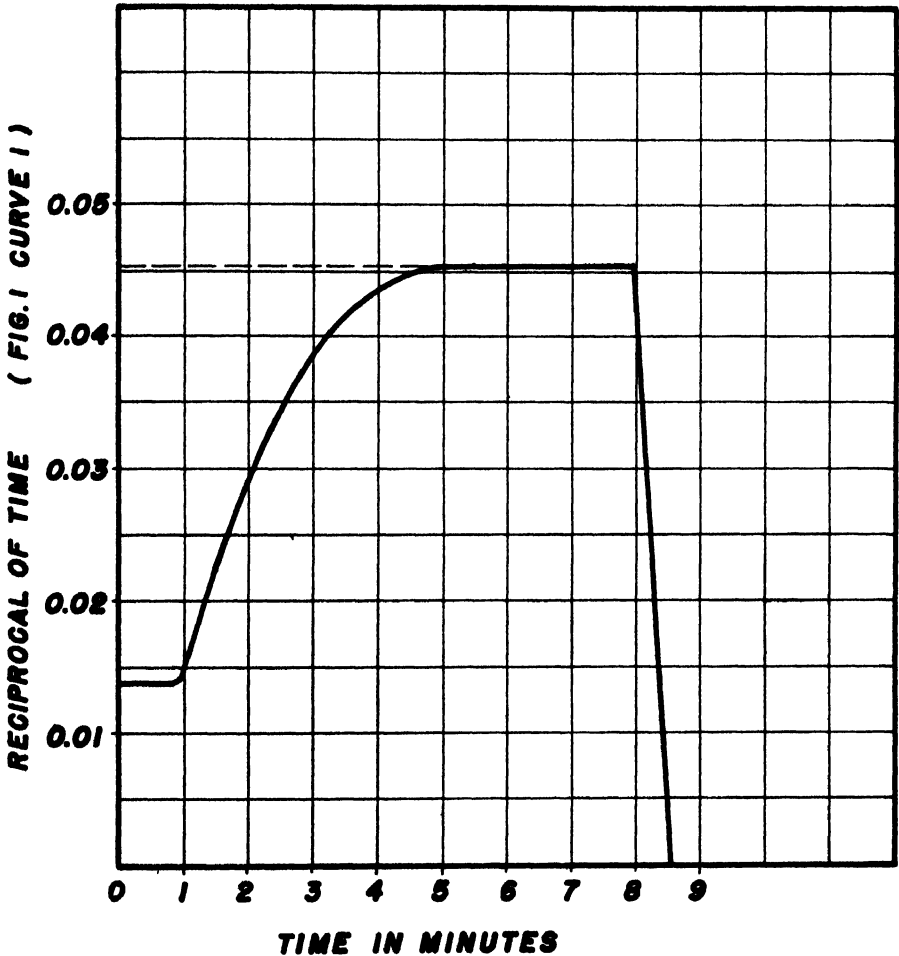


FIG. 3. Conversion of heating and cooling curve time into time at 250° F.

ture. Ten minutes were required to attain 205° F. Evaporation of the milk was conducted under vacuum at a temperature of 125° F., after which the milk was homogenized at 2,500-lb. per square inch and standardized to conform with Federal standard evaporated milk. Samples were filled into 14.5 oz. cans and processed to the point of grain formation. The samples were analyzed; the data are recorded in table 3.

TABLE 3

The effect of cooker process variation on the physical properties of evaporated milk

Group	Sample	Viscosity Mojonnier	% Re- flectance 520 mμ	Grain	Surface tension	Cooker process	
						Time	Temp.
		(75° F.)	(%)		(dynes/cm.)	(min.)	(° F.)
1	1	30	80.3	None	52.5	3.2	260
	2	32	80.0	Sl. film	53.5	3.4	260
	3	46	77.0	**	55.5	3.6	260
	4	46	76.0	***	56.5	3.8	260
1	1	25	79.3	None	52.5	5.3	250
	2	31	78.1	None	55.5	6.0	250
	3	32	76.0	None	56.2	7.0	250
	4	45	73.8	Film	56.5	8.0	250
1	5	56	71.3	***	57.3	9.0	250
	1	28	74.3	None	52.5	12.0	240
	2	36	70.5	None	54.0	15.0	240
	3	55	68.8	Sl. film	56.7	17.0	240
1	4	72	64.8	***	57.0	19.5	240
	1	23	77.8	None	53.0	15.0	230
	2	24	70.3	None	53.5	25.0	230
	3	47	64.5	None	55.5	35.0	230
2	4	83	61.0	Film	57.5	40.0	230
	5	110	60.0	Hy. film	58.6	45.0	230
	1	18	81.7	None	52	1	250
	2	22	80.3	None	52.2	3	250
2	3	27	78.1	None	52.3	5	250
	4	30	77.1	None	52.4	6	250
	5	43	73.0	Trace	55.2	8	250
	6	52	70.5	Film	56.8	9	250
2	7	70	69.0	***	57.8	10	250
	1	17	81.4	None	52.2	1	260
	2	23	81.3	None	52.2	2	260
	3	24	79.3	None	52.5	3	260
2	4	24	78.9	None	52.8	3.4	260
	5	30	77.5	None	53.8	3.8	260
	6	30	77.1	None	54.5	4.0	260
	7	39	75.3	Sl. Film	55.5	4.4	260
2	8	48	74.4	***	56.0	4.8	260
	9	47	73.0	****	56.0	5.5	260
	10	70	67.0	*****	57.8	7.0	260
	1	15	81.6	None	51.3	Pretreatment	
	2	10	81.0	None	50.3	Unsterilized	

*, **, ***, **** and ***** indicate increasing severity of grain formation visible on the back of a spoon.

Attention should be called to the variation in time from the first appearance of grain to a severe grain formation at the various temperatures. At 260° F. the safe working range is not over 0.2 minutes, while at 230° F. it is about 5 minutes.

Samples comprising group 2 were added to show the change in surface tension and color at significant times and temperatures during a process. These data show that a rise in surface tension occurs during the pretreatment period and that more time is necessary to reach a maximum value than would be indicated by the rate of rise at the time of the first appearance of grain.

DISCUSSION

Four curves appear on figure 1. Curve 1 has been described as connecting points of equal surface tension or coagulation, but it is important to note from the

data of table 1 that this curve connects points of nearly equal color. Therefore, it will be found that the position of curves 2, 3 and 4 also indicate the relative color of milk processed as indicated by points along these curves. For example, the color index given in table 2 for the process at 260° F. on curve 3 is 73.6. If a line is drawn through this point parallel to curve 1, it will intersect the ordinates 250° F., 240° F. and 230° F. at 5.5, 9.4 and 16 minutes. The differences in time between these ordinates and the corresponding ones on curve 3 when multiplied by the rate of color formation at the respective temperatures and subtracted from 73.6 agree closely as color indices with the values obtained in the process. (The color index rate varies with concentration of the milk and seasonal factors but it is approximately 1.2 at 250° F., 0.7 at 240° F. and 0.4 at 230° F. for short time periods which are beyond the lag period.) This indicates that the derivation of curve 3 from the heat penetration data is essentially correct. Exception must be noted in those extreme cases involving short time-low temperature pretreatment of the raw milk, followed by a short time-high temperature cooking process. For example, sample 1c in table 2 has a reflectance value of 81.2 which is somewhat higher than the value usually obtained on unsterilized milk. It should be noted also that the very short cooking processes are completed during the lag phase of the color formation. In these cases, the color results are difficult to compute, since small changes in pretreatment of the raw milk, and perhaps other factors, result in somewhat unpredictable color values. This observation is supported by the data appearing in table 3 for processes at 260° F.

Since the surface tension curve based on data for the test tube process coincides approximately with the color curve, the time differences referred to in the preceding discussion of color also indicate the essential differences in coagulation observed for processes at points along curve 3. That is, while visible grain formation is essentially equal along the curves representing the various processes, the surface tension and viscosity are not. It will be noted by the data in tables 2 and 3 that the surface tension, viscosity and color index at the time of grain formation increase as the cooking temperature is decreased. The essential difference between heat coagulation in a static test tube and a can in a cooker is in the time of appearance of visible grain with respect to surface tension. In a test tube, visible grain formation, especially at the lower temperatures, does not appear until some time after the marked rise in surface tension has occurred. In the processing of a can of milk, agitation usually is sufficient to produce a visible grain before a high surface tension value can be reached. That is, there are two phenomena observable in the heat coagulation of evaporated milk—a compact flake or grain type of coagulation and the normal heat coagulation as shown by an increase in viscosity or surface tension. An example of these phenomena is evident in the data for processes at 230° F. and 260° F. (group 1, table 3). For milk heated at 230° F., the surface tension reaches a practical maximum before a grain visible on the back of a spoon forms, but for milk heated at 260° F., a grain forms early in the coagulation period and a severe hard grain forms before a high surface tension or viscosity can be obtained.

The problem commercially seems to resolve itself into finding some means of

decreasing the slope of the grain formation curve without, at the same time, so extending the time of coagulation as to render the milk too dark in color. For example, curve 2, figure 1 has a more favorable slope than curve 3, but the process time represented is too long. It has been the observation thus far that seasonal changes in milk composition have produced greater changes in the character of the coagulation than it has been possible to produce by variation in processing procedure.

In most commercial practice, pretreatment of the raw milk is regulated so that the sterilization processes generally fall within the process range represented by section A on curve 1 of figure 1. Since curve 3 happens to be near the location of the sterility curve and to have practically the same slope, variations in sterilization processes are indicated by curve 3. The physical properties of a given lot of evaporated milk are determined largely by the particular process it is given, represented by some point of the curve. The divergence of curve 3 from curve 1 increases with temperature; therefore, an increase in the temperature of processing is accompanied normally by a decrease in the color of the milk. Unfortunately the improvement in color and flavor are accompanied in general by a decrease in viscosity and fat stability. Until pretreatment procedures of the raw milk are devised to improve the fat stability of the processed milk, commercial evaporated milk processes which result in milk lighter than normal in color cannot be used without the risk of adverse results upon storage of the sterilized milk.

Attention should be called to the fact that if cooker times are plotted instead of the calculated process time, the slopes of the curves are, for most practical purposes, almost identical. This is not surprising, since the lag in the temperature rise largely is offset in the subsequent cooling.

SUMMARY

1. A method for estimating equivalent processes in terms of time and temperature, and with reference to grain point, changes in color and surface tension, has been applied to evaporated milk.
2. Surface tension, to the extent that it is affected by the proteins, is a measure of the degree of coagulation, while grain point is an observable coagulation of fat and protein generally occurring before the maximum surface tension value or coagulation has been attained.
3. For a given sample of milk the color index is a measure of the integrated effect of time and temperature of processing.
4. The curves representing points of equal color and surface tension, grain formation and sterility are logarithmic with respect to time. The differences in the physical properties of the evaporated milk studied are indicated by the location of the processes with respect to these curves.

ACKNOWLEDGMENT

The author is indebted to P. C. Wilbur, A. E. Pech and C. R. Stumbo for their valuable suggestions and criticism; to Tom Mansfield for use of the heat penetration data and to F. B. Neal for assistance in the pilot plant work.

REFERENCES

- (1) BELL, R. W., CURRAN, H. R., AND EVANS, F. R. Effects of Temperature and Time of Sterilization upon Properties of Evaporated Milk. *J. Dairy Sci.*, **27**: 913-919. 1944.
- (2) BIGELOW, W. D., BOIHART, G. S., RICHARDSON, A. C., AND BALL, C. O. Heat Penetration in Precessing Canned Foods. *Natl. Canners' Assoc. Bull.* **16L**. 1920.
- (3) HOLM, G. E., DEYSHER, E. G. AND EVANS, F. R. The Relationships of Concentration and Time to the Temperature of Coagulation of Evaporated Skim and Whole Milk. *J. Dairy Sci.*, **6**: 556-568. 1923.
- (4) NELSON, V. The Spectrophotometric Determination of the Color of Milk. *J. Dairy Sci.*, **31**: 409-414. 1948.
- (5) WEBB, B. H., AND HOLM, G. E. The Heat Coagulation of Milk. *J. Dairy Sci.*, **15**: 345-366. 1932.

EFFECT OF THE QUICK AND COMPLETE ELIMINATION OF VITAMIN C ON THE DEVELOPMENT OF THE OXIDIZED FLAVORS IN HOMOGENIZED MILK, WITH SPECIAL REFERENCE TO THE ACTION OF DAYLIGHT

E. S. GUTHRIE AND VLADIMIR N. KRUKOVSKY

*Department of Dairy Industry
Cornell University, Ithaca, N. Y.*

Many instances occur of homogenized milk developing quickly an oxidized flavor and it generally is recognized that daylight is a greater factor in the development of the oxidized flavors in homogenized milk than in unhomogenized milk. In a review of oxidized flavors in dairy products, Brown and Thurston (1) recorded several studies on the effect of homogenization and on the action of daylight on the development of these flavors in milk and its products. Many investigators have reported that proper homogenization was one way of preventing the production of the oxidized flavors for at least a week or so and that daylight could cause serious deterioration of the flavor of dairy products, whether or not the milk or cream had been homogenized.

A variety of terms has been used to describe oxidized flavors. In the opinion of the authors, the term "oily" best describes the oxidized flavor caused by daylight or sunshine. Also, from the viewpoint of clearness it should be known that the vitamin C of milk is represented by both ascorbic and dehydroascorbic acids. The latter is the less stable form, being destroyed easily by heat. In the process of eliminating the vitamin C of milk, the ascorbic acid first is oxidized to dehydroascorbic acid, and, secondly, the dehydroascorbic acid is destroyed by the heat of pasteurization. The term "vitamin C" in this paper includes both ascorbic and dehydroascorbic acids. At the time of the publication of the review of Brown and Thurston (1), the elimination of all the vitamin C in milk as a means of maintaining freshness in dairy products was not known.

One of the summary statements of the first article of this series (2) by the authors is: "The reaction which produces the tallowy flavor could be inhibited by quick and complete photochemical or chemical oxidation of ascorbic acid in the milk to dehydroascorbic acid prior to its pasteurization and storage. Partial oxidation of ascorbic acid stimulates the development of the tallowy flavor." This paper reports a continuation of that study.

This research was concerned with the effect of rapid and complete oxidation of vitamin C and the partial oxidation of vitamin C prior to pasteurization and homogenization on the development of the oxidized flavors. Various factors, such as different homogenization pressures, the complete oxidation of vitamin C of milk by sunlight, a partial oxidation of vitamin C of milk by sunlight, the addition of vitamin C to milk from which the original vitamin C had been oxidized, and the catalytic action of copper were studied.

Received for publication April 8, 1949.

PROCEDURE

The milk in each experiment of this study was fresh, mixed herd milk obtained from ten 10-gallon cans of morning's milk from the Cornell University herd. The milk was pasteurized by heating to 143° F. for 30 minutes. It was stored in brown glass bottles at 0 to 5° C. The tables in this paper represent many sets of similar data.

RESULTS

In the experiments represented in table 1, copper and ascorbic acid, either alone or together, were added to different portions of milk. The milk was exposed

TABLE 1

Effects of complete oxidation of ascorbic acid in milk by daylight prior to pasteurization and homogenization and of the subsequently added ascorbic acid and copper on the development of the oxidized flavors in homogenized milk

Homogeniza- tion pressure	Storage period	Flavor criticisms of:		
		Milk depleted of its total vitamin C (A)	Milk A + 20 mg. ascorbic acid/l. ^a (B) ^a	
			Cu added ^b	Control
(lb.)	(d.)			
Control	1			oxidized
	2			very oxidized
	7			very oxidized
No pressure	1			very oxidized
	2-7			oxidized
	1			very oxidized
500	2-7			?
	1			?
1,000- 3,000	2-7			slightly oxidized to oxidized
	1-7			

^a Commercial ascorbic acid was added within a few hours after the natural ascorbic acid was oxidized.

^b 0.1, 0.2 and 0.3 mg. Cu added per l. of milk.

to sunlight for 25 minutes, pasteurized and then homogenized at the pasteurization temperature. The "no pressure" samples were taken from the milk that was forced through the homogenizer without pressure, whereas the "control" milk was a portion removed prior to homogenization. Certain parts of the homogenizer, particularly the valves, contain copper. The catalytic action of this copper may account for the differences in flavor in the "control" and "no pressure" samples in table 1, the control sample being the one without copper and the one without an oxidized flavor. Development of the oxidized flavors merely was retarded at 500 lb. pressure, whereas it was prevented completely at the higher pressures. The most important observation is that when the milk was depleted of all vitamin C, the oxidized flavors did not develop even when copper had been added, they quickly became intense in the unhomogenized milk, developed to a slight intensity

in milk homogenized at 500 lb., and did not develop in the milk homogenized at 1,000 lb. and above.

Likewise, a study was made of the quick-partial elimination of ascorbic acid in homogenized milk by sunlight on the development of the oxidized flavors. In table 2, the milks that were homogenized at pressures of 1,000 lb. or higher and

TABLE 2

Effect of a quick-partial oxidation of ascorbic acid in homogenized milk produced by sunlight on the development of oxidized flavors (14.5 mg. ascorbic acid/l. milk after homogenization)

Homogeniza- tion pressure	Storage period	Flavor criticisms of:			
		Milk unexposed to sunlight		Milk exposed to sunlight for 7 min. ^a	
		Control	Cu added ^b	Control	Cu added ^b
(lb.)	(d.)				
Control	1		sl. oxidized		very oxidized
	3-6		very oxidized	very oxidized	very oxidized
No pressure	1		oxidized	sl. oxidized	very oxidized
	3-6	oxidized to very oxidized		very oxidized	
500	1-6	slightly oxidized		oxidized to very oxidized	very oxidized
1,000 and 1,500	1-6			oxidized to very oxidized	very oxidized
2,000 and 3,000	1-6				very oxidized

^a At the end of exposure for 7 min. and after pasteurization, the milk contained 6 mg./l. ascorbic acid.

^b 0.1 mg. copper/l. was added immediately after the milk was pasteurized, exposed to daylight, and put into the sample bottles.

were unexposed to sunlight did not develop the oxidized flavors. The data on the milk exposed to sunlight show that the oxidized flavors became pronounced even when only 1 day old, under all pressures.

In the experiments summarized in table 3 the milk was homogenized at 2,000 lb. pressure at the beginning of pasteurization. The pasteurization process was completed after homogenization. The exposures to sunlight in this experiment were 20 and 40 minutes, in contrast to 7 minutes in table 2. When milk containing 18 mg./l. of ascorbic acid was homogenized at 2,000 lb. pressure, the oxidized flavors were not produced. This was true of both normal milk and normal milk plus copper. When those milks that were homogenized at 2,000 lb. pressure were exposed to sunlight for 20 minutes, the metallic flavor soon became very pronounced. Exposure of the milk to sunlight for 20 minutes was sufficient to lower the ascorbic from 18 to 3 mg./l. Action of the sunlight for 40 minutes completely oxidized the ascorbic acid and there were no oxidized flavors. However, there was a slight "daylight" flavor which may have been due to over-exposure. When 19 mg./l. of ascorbic acid were added to a portion of milk from column C, the oxidized flavors appeared as in column B which contained the normal ascorbic acid of milk. In this experiment in which the pasteurization process was completed

TABLE 3

Effects of a quick-partial, and complete oxidation of ascorbic acid in homogenized milk by sunlight, and of the subsequently added copper on the development of oxidized flavors in homogenized milk^a

	Unexposed to light A		Exposed to sunlight				Ascorbic acid added to C D	
			20 min. B		40 min. C			
	Ascorbic acid (mg./l.)	Flavor criticisms	Ascorbic acid (mg./l.)	Flavor criticisms	Ascorbic acid (mg./l.)	Flavor criticisms	Ascorbic acid (mg./l.)	Flavor criticisms
Prior to exposure	18.0	—	18.0	—	18.0	—	0.0	—
After exposure	—	—	3.0	—	0.0	—	19.0	—
After storage for:								
1 d.	15.3	—	0.0	sl. met. ^b	0.0	vsd	16.0	very met.
2 d.	12.2	—	0.0	very met.	0.0	?	13.0	met.
3 d.	7.0	—	0.0	very met.	0.0	?	8.4	sl. met.
7 d.	0.0	—	0.0	very met.	0.0	?	0.0	met.
1 d. ^c	8.0	—	0.0	very met.	0.0	?	7.3	very met.
2-7 d. ^c	0.0	—	0.0	very met.	0.0	?	0.0	very met.

^a The milk was homogenized at 2,000 lb. pressure.

^b Sl. = slightly; met. = metallic; vsd = very slightly daylight; — = good; ? = questionable.

^c Copper added — 0.1 mg./l.

after homogenization, the results were similar to those of the first two experiments when pasteurization preceded homogenization.

SUMMARY

1. Milk alone, or in the presence of copper, did not develop the oxidized flavors in 7 days when homogenized at pressures of 1,000 lb. or above.

2. Milk from which the vitamin C had been oxidized completely by sunlight, with or without copper, did not develop the oxidized flavors in 7 days in either the homogenized or unhomogenized samples.

3. The addition of commercial vitamin C to milk from which the original vitamin C had been eliminated resulted in oxidized flavors like those produced in the samples from which the original vitamin C had not been eliminated.

4. The development of the oxidized flavors as affected by the different factors in this study is the same when pasteurization follows homogenization as when it precedes homogenization.

5. Milk from which the vitamin C had been oxidized partially by sunlight, with or without copper, quickly developed a "very oxidized" flavor in both the homogenized and the unhomogenized samples.

REFERENCES

- (1) BROWN, W. C., AND THURSTON, L. M. A Review of Oxidation in Milk and Milk Products as Related to Flavor. *J. Dairy Sci.*, **23**: 629-685. 1940.
- (2) KRUKOVSKY, V. N., AND GUTHRIE, E. S. Ascorbic Acid Oxidation, a Key Factor in the Inhibition or Promotion of the Tallowy Flavor in Milk. *J. Dairy Sci.*, **28**: 565-579. 1945.

THE PASTEURIZATION OF AMERICAN CHEDDAR CHEESE BY RADIO-FREQUENCY HEAT

F. V. KOSIKOWSKY, B. L. HERRINGTON AND A. C. DAHLBERG

Department of Dairy Industry, Cornell University, Ithaca, New York

Radio-frequency or electronic heating has found many applications in heavy industry. However, only in recent years has attention been directed to its application in the food industry. Experimental studies have shown that it is possible to cook meat (1), blanch vegetables (8) or heat bread (2) directly in the package. Each of these applications has shown promise, while at the same time presenting new problems.

According to Kinn (3) radio-frequency heating may be defined as the generation of heat in a material normally considered an electrical insulator when that material is placed in a varying electrostatic field. Heating may or may not take place very quickly and uniformly, depending upon the character of the material treated.

The material is inserted between two plates (or electrodes) and an alternating potential is applied. One electrode has a positive charge and the other a negative charge at any given instant and the electrostatic field between the plates causes a deformation of the molecular structure in the material. As a result of the high frequency alternation of the electric charge, the molecules are deformed repeatedly and a molecular stress or friction is set up. This friction becomes apparent in the formation of heat generated throughout the material. By increasing either the frequency or voltage, the movements of the molecular structure are increased and more heat is generated.

Milk has been pasteurized by Brown *et al.* (1) using radio-frequency heat, but, insofar as known, no attempt has been made to pasteurize cheese in this manner, though a preliminary report was presented on this subject by the authors in 1948 (7).

The purpose of this investigation was to pasteurize cheese to various temperatures using radio-frequency heat and to observe the effect of this heat treatment upon the physical nature of the cheese, upon the bacteria and phosphatase present and upon the curing qualities of the cheese.

EXPERIMENTAL METHODS

A number of batches of raw milk were made into American Cheddar cheese, which were pressed overnight in "20-lb." square hoops. These cheeses then were cut up into blocks ($1.5 \times 4 \times 5.25$ inches), packaged in Parakote and heated directly by placing between the two electrodes of an experimental R.C.A. radio-frequency oscillator having a possible power output of 750 watts at 150 megacycles. The time required for heating a 1.3-lb. block of cheese to the desired temperature ranged from 1.5 to 2.7 minutes. Temperatures to which cheeses were heated ranged from 117° to 155° F. After attaining the desired temperatures.

Received for publication April 11, 1949.

the cheeses were placed in cardboard boxes and left in the laboratory to cool. Mercury thermometers were used, being inserted in the centers and sides of the cheeses. The interior temperature of the cheese was maintained for about 30 minutes after removing from the machine.

Analytical methods included the standard plate count as earlier used on cheese by Kosikowsky and Dahlberg (4), the soluble protein by the method of Sharp (11) and the volatile fatty acids by the method of Kosikowsky and Dahlberg (5). In addition, the phosphatase results were obtained by the method of Sanders and Sager (9) and also by a new method based upon modification of the Kay and Graham method (6). Four experienced judges scored the cheeses.

Cheeses shown in this study do not represent the full number tested, for in the interest of simplicity only one representative group is shown.

EXPERIMENTAL RESULTS

Physical appearance of heated cheeses. Two-day-old raw milk cheeses held at 50° F. and heated to as high as 146° F. by the high frequency heater retained their physical form. There was no oiling off and, after cooling to 60° F., there was no notable difference apparent between the heated and unheated samples. However, if the raw milk cheese was held for 10 days at 50° F. and then heated by means of the radio frequency heater, the cheese could be heated to only about 135° F. before losing its physical shape. Above this point, the temperature of the surface continued to rise, while that of the center did not. Under these conditions the surface soon melted. This phenomenon was more evident in cheeses 6 months to a year old, where even lower heating temperatures were necessary. More uniform heating was produced by heating the cheese directly in the Parakote package than by heating the wrapped cheese in a cardboard box.

TABLE 1

The bacterial counts of American Cheddar cheeses^a heated to various temperatures with radio-frequency heat

Heat treatment of cheese	Bacterial count/g. 24 hr. after heating	% Destruction	Bacterial count/g. after 2 mo. at 60° F.	Bacterial count/g. after 6 mo. at 60° F.
(° F.)				
None	320,000,000		51,000,000	1,800,000
126	120,000	99.96	9,000,000	3,200,000
132	5,000	99.99	7,800,000	1,800,000
134	13,000	99.99	9,000,000	880,000
138	3,500	99.99	6,000,000	1,200,000
140	2,000	99.99	6,300,000	6,600,000
146	1,500	99.99	3,100,000	6,600,000

^a Moisture of control cheese = 36.0%; pH of raw cheese = 5.2.

Bacterial count and phosphatase values of heated cheeses. In table 1 it may be seen that heating the cheese by radio frequency heat to a temperature of only 132° F. reduced the total count by 99.99 per cent. However, 2 months later at 60° F. these same cheeses showed an increase in their bacterial count, indicating

that growth had taken place in the meantime. In 6 months (3 months at 60° F. and 3 months at 36° F.) this bacterial count still was maintained in some cases, but in others it had dropped. The types of bacteria growing in this cheese were not investigated.

TABLE 2

The phosphatase values of American Cheddar cheese heated to various temperatures with radio-frequency heat

Heat treatment of cheese	Phosphatase Values		Cheese negative or positive
	Sanders-Sager method ^a	Modified Kay-Graham method ^b	
(° F.)	(γ phenol/ 0.25 g.)	(mg. phenol/ 0.5 g.)	
None	40.0	0.624	+
126	9.0	0.063	+
132	1.0	0.006	-
134	2.0	0.012	-
138	1.0	0.009	-
140	1.0	0.006	-
146	1.0	0.000	-

^a Value over 3.0 γ /0.25 g. cheese indicates underpasteurized or raw milk cheese.

^b Value over 0.02 mg./0.5 g. cheese indicates underpasteurized or raw milk cheese. Data for this standard yet unpublished.

As may be seen by the results from the phosphatase tests (table 2), when the cheeses were heated to 132° F. and higher, negative results were obtained. This is in good agreement with the work of Sanders and Sager (10) who obtained a negative phosphatase test by heating Cheddar cheese to 130° F. for 13 minutes by conventional heating methods.

Ripening of heated cheeses. All the cheese heated by radio frequency heat ripened to some degree and in a measure dictated by the intensity of the heat treatment. None actually attained the degree of ripening of the raw milk control, but some were well broken down at the end of 6 months. The cheeses at the end of 6 months ranged from mild to medium in flavor intensity.

Some criteria of the degree of ripening can be ascertained by observing the increase in soluble protein and volatile fatty acids (table 3). The raw milk cheese in this table was highest in soluble protein and volatile fatty acids at the end of the ripening periods. However, curing had taken place in the heated cheese, as evidenced by the varying increases in these two constants.

Important criteria of ripening are body breakdown and flavor characteristics. Data obtained by four judges on this phase at the end of 2 months are shown in table 4. After two months at 60° F. for this particular lot of cheeses, the flavor score ranged from 38.5 to 39.9, indicating that the cheese flavor was of good quality. The body score ranged from 27.6 to 29.3, a wider variation than that obtained on flavor. The more numerous criticisms referred to flavor at the end of 2 months were slightly bitter and slightly oily, while the body was criticized mostly for being too corky and firm. However, when the same cheeses were

scored again by two judges at the end of 6 months (3 months at 60° and 3 months at 36° F.), the cheeses had deteriorated in flavor but most had improved in body. Most of the criticisms on flavor were bitter and tallowy, while the only criticism on body was slight firmness.

TABLE 3

The soluble protein and volatile fatty acid values of American Cheddar cheese heated to various temperatures with radio-frequency heat and ripened

Heat treatment of cheese	Soluble protein ^a		Volatile fatty acids ^b	
	After 2 mo. at 60° F.	After 6 mo.	After 2 mo. at 60° F.	After 6 mo.
(°F.)	(%)	(%)	(ml. 0.1 N acid/100 g. cheese)	
None	5.95	8.15	35.7	43.9
126	4.93	7.50	28.5	41.7
132	5.09	6.30	27.9	36.2
134	4.74	6.88	20.3	24.0
138	4.51	5.57	19.1	27.0
140	4.70	7.16	27.7	31.0
146	4.51	5.90	17.6	31.1

^a Soluble protein of control cheese 24 hr. after making = 1.29%.

^b Volatile fatty acids of control cheese 24 hr. after making = 12.5 ml. 0.1 N acid.

^c Cured 3 months at 60° F. followed by 3 months at 36° F.

TABLE 4

The flavor, body and total scores of American Cheddar cheese heated to various temperatures with radio-frequency heat and ripened for 6 months

Heat treatment of cheese ^a	Cheese ripened for 2 mo. at 60° F.			Cheese ripened for 6 mo. (3 mo. at 60° F. and 3 mo. at 36° F.)		
	Flavor score ^b	Body score ^c	Total	Flavor score ^b	Body score ^c	Total score
(°C.)						
None	39.1	29.1	93.2	38.3	29.3	92.6
126	39.9	29.3	94.1	37.3	29.3	91.6
132	39.6	29.3	94.0	37.5	29.3	91.8
134	39.0	28.8	92.5	37.0	29.0	91.0
138	38.8	28.8	93.0	37.8	29.0	91.8
140	39.5	28.5	93.2	36.5	29.0	90.0
146	38.5	27.6	91.1	36.8	29.0	90.3

^a Cheese 2 d. old when heated.

^b Most frequent flavor criticisms: oily, bitter.

^c Most frequent body criticism: firmness.

Heating of Camembert cheeses. A number of 8-ounce whole Camembert cheeses were heated with radio frequency heat. These cheeses behaved like aged Cheddar cheese, as when the Camembert wheels were heated to between 60 and 90° F. in the center, it was impossible to handle the surface because of the extremely high temperature. For this reason these cheeses were placed in the heater for time intervals ranging from 0.5 to 2 minutes, instead of measuring temperatures. Only a limited number of cheeses were heated in this manner.

Those heated for 1 to 1.5 minutes were found by various judges to be less prone to have their surfaces turn brown or to have their flavors become ammoniacal after storing at 60° F. than the unheated control sample. No off-flavors developed. However, in time all the cheeses became brown and overripe.

DISCUSSION

It would be advantageous to develop flavor in raw milk cheese before pasteurizing it, but this does not seem possible under the conditions of this study, as rapid melting of the older cheese occurred. Recognition should be made of the off-flavors, such as oily or bitter, which developed in many of the heated cheeses upon prolonged ripening. The source of these off-flavors is not known, though it may be that the oily flavor has something to do with the actual heating, whereas the bitter flavor may be due to bacteria in the cheese. In the control cheese, for example, a bitter flavor was noticed. It might be possible to have *Streptococcus faecalis* as the predominating bacterial flora in the heated cheese by adding large amounts of this thermoduric organism to the cheese milk. This might provide more uniform ripening with good Cheddar flavor.

In the manufacture of Camembert cheese it would be advantageous to delay the ripening process of the cheese after it had reached its optimum point. A very limited amount of work was done on this problem and the preliminary results indicate some delay is brought about, although only rough methods of estimating temperatures were used.

Care should be taken in the heating of ripened cheese that arcing of the electrodes does not result, for brown discolorations appeared on the cheese if this happened. Proper shaping of the electrodes for the cheese is essential.

Many problems exist in this type of heating, aside from economic questions. For example, no good means of determining the temperature during the heating process is available, nor have those factors been studied which are likely to cause irregularities in temperature throughout the cheese. More information is necessary in regard to proper development of flavor and body, and especially the production of off-flavors. After this information is obtained, the careful standardization of time-temperature relationships will be necessary to assure proper pasteurization. It is felt that more information of this nature should be obtained before the practicability of this type of pasteurization can be evaluated fully.

SUMMARY

The radio-frequency pasteurization of young Cheddar cheese in Parakote packages was found possible. Older cheeses did not stand up well under the heat treatment. Phosphatase negative results were obtained on Cheddar cheese heated by radio frequency currents to 132° F. or above, and then cooled in air.

Curing took place in radio frequency heated cheeses, though not as rapidly as in the unheated controls. Off flavors, including oily and bitter, were noticed in a number of heated cheeses. Their cause was not investigated.

Many problems are present in this type of heating which require additional study.

ACKNOWLEDGMENT

This investigation was aided by a grant from the National Cheese Institute. The authors are indebted to Mrs. Catherine Verwoert Work for her aid in making many of the chemical analyses and to W. E. Ayres and H. B. Ayres and H. B. Naylor for their assistance in scoring the cheeses.

Acknowledgment also is made of the technical advice so generously given by J. C. Moyer of the New York State Agricultural Experiment Station and by George Warfield of the Physics Department of Cornell University.

Finally, the authors wish to thank the R.C.A. Laboratories for the loan of the radio-frequency oscillator used in this work.

REFERENCES

- (1) BROWN, G. H., HOYLE, C. N., AND BIERWIETH, R. A. *Theory and Application of Radio-frequency Heating*. D. VanNostrand Co., Inc., New York, N. Y. 1947.
- (2) CATHCART, W. H., PARKER, J. J., AND BEATTIE, H. G. The Treatment of Packaged Bread with High Frequency Heat. *Food Technol.*, 1: 174-177. 1947.
- (3) KINN, T. P. Basic Theory and Limitations of High Frequency Heating Equipment. *Food Technol.*, 1: 161-173. 1947.
- (4) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Growth and Survival of *Streptococcus faecalis* in Pasteurized Milk American Cheddar Cheese. *J. Dairy Sci.*, 31: 285-292. 1948.
- (5) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Rapid Direct-distillation Method for Determining the Volatile Fatty Acids of Cheese. *J. Dairy Sci.*, 29: 861-871. 1946.
- (6) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. The Elimination of Interfering Substances in the Kay-Graham Phosphatase Test when used for Hard Ripened Cheese. *J. Dairy Sci.*, 31: 561-567. 1948.
- (7) KOSIKOWSKY, F. V., HERRINGTON, B. L., AND DAHLBERG, A. C. A Preliminary Note on the Pasteurization of American Cheddar Cheese by Radio-Frequency Heat (Abstract). *J. Dairy Sci.*, 31: 710. 1948.
- (8) MOYER, J. C., AND STOTZ, E. The Electronic Blanching of Vegetables. *Science*, 102 (2639): 68-69. 1948.
- (9) SANDERS, G. P., AND SAGER, O. S. Modification of the Phosphatase Test as Applied to Cheddar Cheese and Application of the Test to Fluid Milk. *J. Dairy Sci.*, 29: 737-749. 1946.
- (10) SANDERS, G. P., AND SAGER, O. S. Present Status of the Phosphatase Test. *J. Milk and Food Technol.*, 11: 67-75. 1948.
- (11) SHARP, P. F. Private communication, about 1940, as reported by Dahlberg, A. C., and Kosikowsky, F. V. The Flavor, Volatile Acidity and Soluble Protein of Cheddar and other Cheese. *J. Dairy Sci.*, 30: 165-174. 1947.

TABLE 1
Effect of the method of drying cow manure on its male hormone activity

Method of drying	Av. drying temp.	Drying time	Male chicks			Female chicks			Male and female	
			No. of chicks	Av. body wt.	Comb wt./100 g. body wt.	No. of chicks	Av. body wt.	Comb wt./100 g. body wt.	Comb wt./100 g. body wt.	Comb wt./100 g. body wt.
	(°C.)	(hr.)		(g.)	(mg.)		(g.)	(mg.)		(mg.)
Dried at 45° C.	45	48	23	248.7	105.19	37	238.5	89.34		97.26
Sun-dried	34 ^a	96	8	250.3	63.92	11	232.7	37.29		50.60
Air-dried	28 ^a	192	8	287.3	39.63	12	265.2	22.54		31.08
Fan-dried	27 ^a	72	9	239.2	92.08	11	245.9	76.21		84.14
Air- and fan-dried	23 ^a	144	10	288.9	44.14	10	266.4	29.69		36.91
Quick-dried	45	17	10	241.1	47.82	12	200.2	37.42		42.62
Fresh manure			7	252.9	44.80	11	214.1	22.83		33.81
Dried in small lots	45	48	15	230.3	89.15	6	215.3	53.96		71.55
Acidified	45	48	9	262.4	55.35	12	257.2	27.82		41.58
Dry, wet and re-dry at 45° C.	{ 45	48	12	241.9	58.26	9	232.9	34.75		46.50
	{ 45									
Dry at 45° C., wet, dry at 80° C.	{ 45	72	22	240.3	114.55	16	239.4	84.91		99.73
	{ 80	24								
Dry at 45° C., heat at 80° C.	{ 45	72	24	257.8	131.08	15	238.3	93.72		112.40
	{ 80	24								
Dry at 80° C.	80	24	20	251.8	38.14	19	257.9	29.27		33.70
Control group			11	264.5	42.12	10	248.0	26.16		34.14

^a Av. noon temperature at time of drying.

TABLE 2
Effect of various treatments of fresh cow manure

Treatment of manure fed at 10% equiv. level	Male chicks			Female chicks			Male and female	
	No. of chicks	Av. body wt. (g.)	Comb. wt. 100 g. body wt. (mg.)	No. of chicks	Av. body wt. (g.)	Comb. wt. 100 g. body wt. (mg.)	Comb. wt. 100 g. body wt.	(mg.)
Fresh manure	10	228.9	27.11	10	207.9	15.85		21.48
Heated 3 hr. at 45° C., wet	10	226.3	29.95	11	207.7	23.79		26.87
Heated 14 hr. at 45° C., wet	13	220.7	30.18	8	187.4	17.36		23.77
Heated 24 hr. at 45° C., wet	13	236.8	30.21	8	197.1	25.18		27.70
Heated 48 hr. at 45° C., wet	11	226.3	30.85	10	202.7	17.13		23.99
Control feed, no manure	10	219.4	35.89	10	202.4	21.40		28.65
Heated 15 lb. pressure, dried at 45° C.	8	241.9	84.50	12	219.8	57.90		71.20
Heated 15 lb. pressure, dried at 45° C., wet	8	187.9	60.94	14	186.6	67.68		64.31
Heated 15 lb. pressure, fed wet	5	198.8	53.15	14	215.9	28.81		40.98
Fresh manure	8	183.0	42.64	11	181.4	86.61		92.91
Dried at 45° C.	11	208.7	97.81	9	193.3	33.83		38.24
Dried at 45° C., wet	10	211.2	86.73	9	180.6	91.19		88.96
Dried at 80° C.	7	212.1	48.82	12	209.8	40.05		44.44
Dried at 80° C., wet	7	212.1	50.89	10	194.4	36.68		43.79
10 mg. Testosterone/kg. feed	7	249.9	134.87	12	201.3	96.23		115.55

Since it is somewhat difficult to picture the drying process causing the hydrolysis of combined androgen, the feeding of fresh manure was compared with similar material heated in an autoclave at 15 lb. pressure for 25 minutes, then cooled and fed wet. The inactivation of the enzymes and microorganisms was without beneficial effect in the feeding of wet manure (table 2). Further, the high temperature treatment (121° C.) was ineffective as a hydrolyzing agent when the hormone was present in a combined form. If the cow manure, after heating at 15 lb. pressure for 25 minutes, was dried at 45° C. and then fed, or if rewet after drying, the biological activity was increased considerably, but it showed less potency than the manure dried at 45° C. without autoclaving. The detrimental effect of drying at 80° C. again was shown. Wherever the feeding of the material in the dry and wet states was compared, the dry material was slightly more effective.

For comparison with the results obtained with cow manure dried at 45° C., a group of chicks was fed 10 mg. testosterone per kilogram of feed. The crystalline hormone at this level was slightly more potent than the hormone present in the manure when dried at 45° C.

In the first experiment, reheating at 80° C. for 24 hours one sample of cow manure which had been dried at 45° C. appeared to increase the biological activity of the male hormone present (table 1). To confirm this work and at the same time determine the optimal temperature for reheating, manure was collected from a single cow for a considerable period and dried at 45° C. by the regular method. When sufficient material was on hand, it was divided into five lots. The first lot was not reheated, but the second to fifth lots were heated for 24 hours at 65, 85, 105 and 125° C., respectively.

The assay indicated that the apparent androgen content of this sample of cow manure dried at 45° C. was slightly higher than that observed in the first experiment and closely approached the average comb weight stimulated by 10 mg. testosterone per kg. feed (table 3). However, by redrying the samples at 65° C. for 24 hours, the average comb weight per 100 g. body weight was increased to 170.44 mg. which compares quite favorably with the average comb weight of 183.59 mg. stimulated by 20 mg. testosterone per kg. feed (3). Heating the dried cow manure at progressively higher temperatures then caused a gradual decline in the biological activity of the male hormone. The final temperature, 125° C., greatly reduced the activity.

DISCUSSION

These data indicate that the male hormone in fresh cow manure is present in an inactive form. Heating and drying cow manure at 45° C. increase the biological activity of the hormone present. Heating fresh cow manure at 45° C. for varying time intervals up to 48 hours without drying was without effect upon the biological activity. Autoclaving fresh cow manure at 15 lb. pressure for 25 minutes to inactivate bacteria and enzyme activity was without effect either from the standpoint of retarding unfavorable changes in the hormone or from the possible beneficial effects of the high temperature (121° C.).

TABLE 3
Effect of heat treatment on manure dried at 45° C.

Treatment of manure fed at 10% level	Male chicks			Female chicks			Male and female	
	No. of chicks	Av. body wt.	Comb wt./ 100 g. body wt. (mg.)	No. of chicks	Av. body wt.	Comb wt./ 100 g. body wt. (mg.)	Comb wt./ 100 g. body wt.	(mg.)
Dried at 45° C.	8	214.9	110.91	12	212.9	103.36	107.14	
Redried at 65° C. for 24 hr.	11	226.6	208.26	9	221.0	132.61	170.44	
Redried at 85° C. for 24 hr.	11	228.3	139.06	8	206.6	118.16	128.61	
Redried at 105° C. for 24 hr.	14	239.6	114.67	5	235.8	107.59	111.13	
Redried at 125° C. for 24 hr.	10	218.0	75.95	9	205.0	60.55	68.25	

Apparently desiccation, rather than temperature, was the paramount factor in the biological activation of the hormone resulting from the drying of cow manure at 45° C. for 48 hours. However, slow drying, either in the sun or shade or by fanning at room temperature resulted in samples of reduced potency.

If temperatures of drying the fresh manure were increased above 45° C., biological activity again was reduced. About 45° C. apparently is the optimum temperature for the desiccation of fresh cow manure. When desiccation is about complete, continued heating at about 65° C. greatly increases the oral biological activity of the hormone.

In the metabolism and excretion of estrogen, the natural hormone, estradiol, is changed in part to the less active estrogens, estrone and estriol, and, further, these compounds are combined as sulphates and glucuronidates before being excreted. Since the estrogens in urine in the combined form are less active biologically than in the free form, the hydrolysis of urine either by acid or as a result of the action of microorganisms has resulted in the production of urine showing increased biological activity.

The androgens in urine also have been shown to occur in a water-soluble, biologically inactive form. On treatment with acid and heat, the water-soluble complex is split, yielding fat-soluble, water-insoluble androgen, which is biologically active (1). It has been demonstrated that the combined androgen is in part a sulfate ester.

The lack of biological activity in the fresh manure might be taken to indicate that the male hormones are present in a combined form just as they are secreted in urine. In the case of urine, the hormones are activated by the hydrolysis of the compounds by heat or acid to liberate the free forms. If the same situation exists in the case of the male hormone in cow manure, it would seem that the appearance of the free form of the hormone is accelerated by desiccation at a temperature of about 45° C. When this process is about complete the conversion to the free form of the hormone is further effected by holding at temperatures of about 65° C. for 24 hours. By this treatment, the cow manure of individual cows may be shown to contain male hormone with oral biological activity approaching the biological activity of 20 mg. of testosterone per kg. of feed. Since the dried cow manure is fed at the 10 per cent level, it means that 100 g. of dried cow manure may contain the oral equivalent of 20 mg. of testosterone.

At this time it is not possible to say that the desiccation and temperature conditions which increase the oral biological activity of the cow manure do so by hydrolyzing the combined male hormone. Possibly other changes in the compounds affecting their biological activity might be produced by these physical conditions. Only by the isolation and characterization of the compounds present in cow manure can this problem be solved.

SUMMARY AND CONCLUSIONS

1. Sun- and air-drying were less effective than fanning for drying manure, but none of these methods was as effective as drying at 45° C. for 48 hours.

2. Feeding of fresh manure was without effect, indicating that the male hormone in fresh cow manure is biologically inactive.

3. The previous observation that heating fresh manure at 80° C. inactivated the hormone was confirmed; however, when the manure is dried at 45° C., it may be wet and redried at 80° C. or heated at 80° C. for 24 hours without loss of activity.

4. Heating cow manure at 45° C. without drying for periods varying from 3 to 48 hours was without beneficial effect.

5. Autoclaving the manure at 15 lb. pressure for 25 minutes neither improved the activity when fed moist nor seriously depressed the activity when dried subsequently at 45° C.

6. When manure dried at 45° C. was reheated for 24 hours at 65° C., the apparent activity was almost doubled. Heating at both 85 and 105° C. resulted in activity equal to the control sample, but heating at 125° C. inactivated the hormone rather severely.

REFERENCES

- (1) DORFMAN, R. I. Biochemistry of Androgens. Chapter XII. in *The Hormones, Physiology, Chemistry and Applications*. Vol. 1, p. 467. Academic Press Inc., New York, N. Y. 1948.
- (2) TURNER, C. W. The Male Hormone Content of Ruminant Manure. *J. Dairy Sci.*, 30: 1-8. 1947.
- (3) TURNER, C. W. Some Factors Influencing the Male Hormone Content of Cow Manure. *J. Dairy Sci.*, 31: 1032-1040. 1948.

THE EFFECT OF PREPARTUM MILKING AND OF FEEDING OF VITAMIN A SUPPLEMENTED RATION ON THE LEUCOCYTE COUNT OF POSTPARTUM MILK SAMPLES

E. O. ANDERSON AND PATRICIA MACLEOD

*Department of Animal Industries, Storrs Agricultural Experiment Station,
University of Connecticut, Storrs*

This paper reports the effects of prepartum milking and the feeding of a vitamin A-supplemented ration on the leucocyte counts of postpartum milk samples. An analysis of the effects of these factors on the composition of postpartum milk will be published elsewhere.¹

METHODS

Films of milk samples of the first postpartum milkings of 32 mastitis-free (3) cows were stained and examined by the methods prescribed by the American Public Health Association (1). The cows were milked on a 12-hour schedule. Sixteen were subjected to prepartum milking, beginning 3 days before the calculated parturition date. The actual period of prepartum milking varied from 36 hours to 9 days. Of these cows, milked prepartum, five were fed the basal ration and 11 the same basal ration supplemented by vitamin A. The other 16 animals were not milked before parturition. Four of these were fed the basal ration and 12 the supplemented diet.

The leucocyte counts, expressed in millions of cells per ml. were converted to logarithms for statistical analysis. The milk samples of the four differently-treated groups of animals were compared in respect to both the mean leucocyte count and the change in the leucocyte count during the first six milkings postpartum.

RESULTS

The average leucocyte counts of the four groups differed less from one another than the error of the difference. The average log-counts of the prepartum basal and vitamin A groups were 5.76 and 5.84, respectively, and those for the two groups milked only postpartum were both 5.77. In actual counts these correspond to the geometric means shown in the last row of table 1, together with the average amount of milk produced, in pounds.

The number of leucocytes decreased strikingly and very significantly during the six postpartum milkings when averaged over all four groups. This downward trend was somewhat less marked in the prepartum-milked cows than in the others, although the difference in the trend could not be considered as statistically significant. The addition of vitamin A to the ration had no effect upon the trend.

Keyes *et al.* (2) found that the number of leucocytes was the highest in the

Received for publication April 29, 1949.

¹ This study is being conducted by H. D. Eaton, Department of Animal Industries, University of Connecticut.

TABLE 1

Average milk production and leucocyte counts of postpartum milkings, for the four groups of experimental cows

Postpartum milking	Prepartum milked cows ^a				Non-prepartum milked cows ^b			
	Basal ration		Vitamin A Ration		Basal ration		Vitamin A Ration	
	Leucocytes	Milk	Leucocytes	Milk	Leucocytes	Milk	Leucocytes	Milk
	(No./ml.)	(lb.)	(No./ml.)	(lb.)	(No./ml.)	(lb.)	(No./ml.)	(lb.)
1st	676,000	12.26	1,000,000	9.99	1,122,000	14.00	1,202,000	14.53
2nd	851,000	16.58	1,175,000	12.35	2,042,000	7.88	1,112,000	8.37
3rd	537,000	16.92	562,000	15.23	490,000	14.55	617,000	10.69
4th	513,000	19.68	525,000	16.67	355,000	17.38	427,000	14.68
5th	479,000	17.62	555,000	16.77	229,000	17.68	407,000	14.19
6th	490,000	20.68	550,000	15.33	263,000	17.08	302,000	17.25
Av. for group	575,000	17.29	692,000	14.39	589,000	14.76	589,000	13.29

^a 16 cows: 5 on basal ration (1 Ayrshire, 1 Guernsey, 2 Holsteins, 1 Jersey); 11 on vitamin A ration (4 Guernseys, 3 Holsteins, 4 Jerseys).

^b 16 cows: 4 on basal ration (2 Guernseys, 1 Holstein, 1 Jersey); 12 on vitamin A ration (2 Ayrshires, 3 Guernseys, 4 Holsteins, 3 Jerseys).

first milkings and dropped to normal (the actual number was not given) within 4 days after parturition.

CONCLUSION

The mean leucocyte count of milk samples of postpartum milkings of healthy cows is not affected by prepartum milking or by the feeding of a vitamin A-supplemented ration.

ACKNOWLEDGMENT

The authors wish to express their appreciation to C. I. Bliss, Biometrician, Storrs Agricultural Experiment Station, for his help in the statistical analysis of the data, and to H. D. Eaton, Department of Animal Industries, University of Connecticut, for the milk production figures.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. *Standard Methods for the Examination of Dairy Products*. 8th ed. New York, N. Y. 1941.
- (2) KEYES, E. A., REID, J. J., BECHDEL, S. I., BORLAND, A. A., BEAM, A. L., AND WILLIAMS, P. S. Pre-Partum Milking (Abstr.). *J. Dairy Sci.*, **27**: 638-639. 1944.
- (3) PLASTRIDGE, W. N., ANDERSON, E. O., WILLIAMS, L. F., AND HALE, H. H. Infectious Bovine Mastitis. 9. Information on the Control of Chronic Mastitis. Storrs (Conn.) Agr. Expt. Sta. Bull. **255**. 1946.

OCCURRENCE OF PROTOZOA IN THE BOVINE STOMACH¹

EARLE M. UZZELL, R. B. BECKER, AND E. RUFFIN JONES, JR.

University of Florida, Gainesville

Digestion of feeds by cattle is accomplished mechanically, by enzyme action, by chemical reactions and through the activity of microorganisms, such as the bacteria and protozoa in the stomach (23). Some attention has been given to these protozoa, yet much remains to be learned concerning their kinds, distribution and activity.

LITERATURE REVIEW

In 1843, Gruby and Delafond (11) observed large numbers of microorganisms, including ciliate infusoria, in the rumen and reticulum but only dead specimens or fragments in the omasum, abomasum and small intestines. Hastings (13) quoted estimates placing the volume of protozoa at 4.4 to 20.0 per cent of the rumen contents.

Becker and Talbott (6) listed 33 species of protozoa as having been identified in the rumen, with from 2 to 16 species present in single animals. Mangold (22) mentioned 19 species of protozoa obtained from the stomachs of cattle out of 33 reported for eight different ruminants. These microorganisms function in digestion and synthesis of nutrients in the rumen and become available to the host when they, in turn, are digested.

Becker and associates (4, 5) suggested that the rumen protozoa enter the stomach through ingestion of freshly contaminated feed or water, and that they were not ingested in cyst form. Becker (3) believed that the numbers of protozoa were related more closely to kind and quantity of feed eaten than to physiological state of the animal. Johnson *et al.* (19) observed the concentration of bacteria to be greatest 1 hour after feeding (6.5 million/ml.), and of protozoa 15 hours later (840,000/ml.). However, six lambs defaunated by fasting coupled with two copper sulfate treatments at 24-hour intervals were kept free of rumen protozoa for 30 days. They utilized urea as efficiently as did normal lambs. Functioning of rumen bacteria was regarded as possible but not investigated. Protein from protozoa was 86 per cent digested and that of bacteria, 55 per cent. Biological values of these proteins with rats were 68 and 66 per cent, respectively.

Schwarz (26) investigated rumen contents of slaughtered cattle and concluded that one-third of the protein in feeds was converted into bacterial and infusorial protein. He cited Scheunert's view that infusoria were harmless commensals aiding in the mechanical action along with the soaking, maceration and intermixing of the paunch contents, agreeing with Bündle on this point.

Microorganisms of the stomach (anaerobic bacteria and protozoa) functioned in the digestion of cellulose (15, 19, 21, 22, 28) and in the conversion of urea, ammonium carbonate and vegetable proteins into animal proteins available ulti-

Received for publication April 30, 1949.

¹ A joint contribution of the departments of Dairy Husbandry and Biology. Approved for publication by the director of the Florida Agricultural Experiment Station.

mately to the host. Some products of this activity included cellulose disintegration, gases and ether-soluble compounds, as indicated by Baker (1), Cole *et al.* (7), Hale *et al.* (12) and Hungate (17). Rumen microorganisms also contributed to the synthesis of water soluble vitamins (2, 8, 18, 21, 24, 25), including biotin, pantothenic acid, pyridoxine, riboflavin and thiamine.

Use of a rumen fistula has facilitated investigations of biological functions of microorganisms in cattle. Penstate Homestead Jessie 924062 had a rumen fistula by means of which the rumen contents were sampled by Bechdel *et al.* (2). They found that *Flavobacterium vitarumen* synthesized vitamin B complex in the rumen. Huffman (14) reported that an experimental cow went seriously off-feed upon complete removal of the rumen contents through a fistula upon continued feeding of the same ration. She recovered quickly after fresh rumen contents from a normal steer were placed in her rumen. Rumen digestion in cattle was practically completed 12 hours after eating (12).

Ferber (9) and Ferber and Winogradowa-Fedorowa (10) regarded protozoa as symbionts, observing in sheep and a goat that these organisms built up easily-digested protein in their bodies to be broken down in the omasum and digested. A wether, fasted 102.5 hours, was reduced to a state of "no infusoria" in the rumen. Upon resumption of feeding, the total infusoria increased in 30 days to 1,387/ml. Van Der Wath and Myburgh (27) recently investigated the role of infusoria and bacteria in ruminal digestion with Merino sheep under South African conditions.

Knowledge of bacterial and protozoan function in the ruminant stomach suggests a need for more information concerning the kinds of rumen infusoria in cattle and their distribution. Consequently, a survey was made with animals raised in the station dairy herd to determine the kinds of protozoa present in Jersey cattle of different ages.

METHODS

Samples of stomach contents from the rumino-reticular compartment were taken from six cattle slaughtered commercially and used in testing technics of observation, staining and identification. No protozoa were found in a sample obtained from a high grade Brahman bull. This animal was in reasonable condition and appeared healthy. The sample contained a considerable proportion of fresh citrus pulp and little grass or hay. Nothing is known concerning his previous feeding and history.

Twelve male Jersey calves and one Jersey cow raised in the experiment station dairy herd were studied under feeding conditions shown in table 1.

Each animal was stunned, suspended head downward and bled. The hide was removed, animal eviscerated and samples obtained from the ileum near the distal end, abomasum, omasum and rumino-reticular compartment, in this order. These alimentary contents were collected in sterilized Mason jars or test tubes with little contact with the air. In the Biology laboratory, samples were taken rapidly at room temperature, using clean pipettes to avoid extra exposure to air, and examined under cover slips sealed with plain vaseline. Observations began within

TABLE 1
Protozoa in the stomach of Jersey cattle in Florida

Animal no.	Age in days	Stomach compartment	<i>Callimastix frontalis</i>	<i>Isoytricha ruminantium</i>	<i>Diplodinium bursa</i>	<i>Diplodinium dentatum</i>	<i>Diplodinium denticulatum</i>	<i>Diplodinium ecaudatum</i>	<i>Diplodinium hegnerti</i>	<i>Diplodinium helseri</i>	<i>Entodinium bicarinatum</i>	<i>Entodinium bursa</i>	<i>Entodinium caudatum</i>	<i>Entodinium furca</i>	<i>Entodinium minimum</i>	<i>Eutrichomastix ruminantium</i>	<i>Isoytricha prostoma</i>	<i>Isoytricha ruminantium</i>	<i>Ophryscolex caudatus</i>	<i>Polyplastron multivesiculatum</i>
<i>Prior to nursing</i>																				
347-F	0.5	Rumen																		
		Omasum																		
		Abomasum																		
352-F	1	Rumen																		
		Omasum																		
		Abomasum																		
<i>Fed milk, concentrates and prairie hay</i>																				
338-F	48	Rumen										x ^a			x					
		Omasum										n ^b			x					
		Abomasum													n					
340-F	59	Rumen										x			x					
		Omasum										x			x					
		Abomasum													n					
341-F	70	Rumen	x			x	x		x	x	x	x	x	x	x	x	x			
		Omasum	x			n	n		n	n	x		x	x	x	x	x			
		Abomasum																		
<i>Fed concentrates and prairie hay</i>																				
336-F	80	Rumen							x	x	x	x	x	x	x					x
		Omasum							x	x	x	x	x	x	x					x
		Abomasum						n	n			n	n	n						n
323-F	160	Rumen	x	x								x	x	x	x		x	x		
		Omasum	x	x								x	x	x	x		x	x		
		Abomasum										n	n	n						
322-F	168	Rumen	x	x	x	x		x	x	x			x		x	x	x	x		
		Omasum		x	x	x		x	x	x			x		x	n	n	x		
		Abomasum		n	x	n		n	n	n			n		n	n	n			
315-F	168	Rumen		x	x	x			x		x	x			x					
		Omasum		x	n	x			x		x	x			x					
		Abomasum		n	n	x			x		n	x			x					
313-F	193	Rumen	x						x	x			x		x					
		Omasum	x						x	x			x		x					
		Abomasum	n					n	n			n	n		n					
<i>Fed concentrates and fresh grapefruit pulp</i>																				
319-F	196	Rumen		x			x				x	x	x			x				
		Omasum		x							x	x	x				n			
		Abomasum		n			n				n	n	n				n			
316-F	201	Rumen									x	x	x	x						
		Omasum									x	x	x	x						
		Abomasum																		
<i>Fed concentrates, corn silage and hay</i>																				
58-F	years 4.5	Rumen	x	x		x	x				x		x	x	x	x	x	x	x	
		Omasum	n	n			x	x			x		n	x	x	n	n	n	n	
		Abomasum				n											n	n		

^a x = living protozoa.

^b n = protozoa non-motile (dead?).

10 to 15 minutes of sampling so as to observe the protozoa in the living state insofar as possible. The samples were examined microscopically in the order of collection.

Aliquot amounts were fixed in 10 per cent formalin, in Schaudinn's fluid and in hot Bouin's fluid. The fixed protozoa were stained with precipitated borax-carmin, with standard alum hematoxylin and triosin. Then they were dehydrated in an alcohol series and mounted in balsam for further species identification by means of the keys of Kudo (20) and Becker and Talbott (6).

RESULTS

No protozoa were found in the digestive tract of either newborn calf, 347-F and 352-F. Clear liquid in the abomasum contained squamous epithelial cells. Some cells also were present in the fluid in the rumen and omasum. One calf had licked sand from the floor of his stall. Neither animal had opportunity to nurse prior to slaughter.

No protozoa were observed in the ileum of any of the calves or in the cow.

No calves fed solely on milk were available for this study. Calf 341-F was believed to have obtained some grass shortly before slaughter, as the stomach contents were green in color. Green inclusions were seen in many of these protozoa, including *Entodinium bursa*. The presence of green inclusions in this species is of particular interest, since Hungate concluded (15, 16) that *Entodinium caudatum* did not digest cellulose.

Five calves from 80 to 193 days old were receiving mixed concentrates and upland prairie hay at time of slaughter. Two of these, 315-F and 322-F, were the only animals in which living rumen protozoa were identified in the abomasum. Considerable gas developed in the rumens of these animals during slaughter, sufficient to force some of the rumen liquid through the esophagus. It is highly probable that the living protozoa in the abomasum were an artifact caused by this pressure. As shown in table 1, from 5 to 12 species of protozoa were identified in the rumen contents of these five animals, 313-F, 315-F, 322-F, 323-F and 336-F. Protozoa seen in the abomasal samples from three of them were non-motile (dead?).

In attempting to follow a clue that feeds may affect the fauna of the digestive system, prairie hay was withdrawn from the rations of 316-F for 10 days, and of 319-F for 11 days, and replaced with fresh grapefruit pulp (peel, rag and seed). Only four species of *Entodinium* were found in the rumen of the first animal when slaughtered, while three of these together with the larger *Diplodinium* were identified in the rumen of the second animal. Blades of prairie hay were present in the rumen contents of 319-F even though none had been offered to him for 11 days.

The 4.5-year old Jersey cow 58-F had been removed from a mixed grass pasture 5 days before slaughter and had received the same kind of mixed concentrates and hay as did the calves, in addition to about 20 lbs. of corn silage daily. This cow's rumen contained 12 species of living protozoa, including two of *Diplodinium*, four of *Entodinium*, two of *Isotricha*, and one species each of *Callimastix*, *Dasytricha*, *Eutrichomastix* and *Ophryoscolex*.

Two of the younger calves had only two species of *Entodinium* in the stomach.

The cow and a 5.5-month old calf had 12 different species of protozoa. Eighteen species in eight genera of rumen protozoa were distributed variously among the 11 animals past 6 weeks old. The five species of *Entodinium* each were present in six to nine animals, only two animals possessing all five species. From one to four calves had one or more of six species of *Diplodinium*, none having only this genus. Three animals had both species of *Isotricha*, and a single animal had one species of this genus. Single species of *Callimastix*, *Dasytricha* or *Eutrichomastix* were present in three different animals, the mature cow having all three of them. *Ophryoscolex* and *Polyplastron* occurred only in single individuals. The distribution of protozoa is listed in table 1.

SUMMARY AND CONCLUSIONS

This survey verified that the stomach and small intestines of calves were devoid of protozoa at birth. No observations were made on calves receiving milk alone. Protozoa were teeming in the rumen, some living ones persisted in the omasum, nearly all in the abomasum were non-motile (dead?) and none were intact in the ileum. This suggests that their main activity took place in the first-named compartment and that, after being rendered non-motile, they were digested by the host. More observations are needed concerning effects of diet on the microfauna both as to species and numbers. Calves appeared to acquire more species of protozoa as they advanced in age. Eighteen species of eight genera of protozoa were observed in 12 experimental Jersey calves and a cow studied in this herd.

ACKNOWLEDGMENTS

Harold S. Gertner Co. permitted samples to be taken from the rumens of cattle slaughtered commercially and slaughtered one of the experimental animals. J. H. Wallace supplied the fresh citrus pulp fed to two experimental animals. Herman Sommers did the special feeding of these two experimental animals. Sidney P. Marshall, P. T. Dix Arnold and several student helpers aided in rapid preparation of 12 calves for alimentary samples to be obtained. H. K. Wallace advised on staining procedures. Thanks are extended to all of these.

REFERENCES

- (1) BAKER, F. Normal Rumen Microflora and Microfauna of Cattle. *Nature*, 149: 220. 1942.
- (2) BECHDEL, S. I., HONEYWELL, H. H., DUTCHER, R. A., AND KNUTSEN, M. H. Synthesis of Vitamin B in the Rumen of the Cow. *J. Biol. Chem.*, 80: 231-238. 1928.
- (3) BECKER, E. R. The Present Status of Problems Relating to the Ciliates of Ruminants and Equidae. *Quart. Rev. Biol.*, 7: 282-297. 1932.
- (4) BECKER, E. R., AND EVERETT, R. C. Comparative Growths of Normal and Infusoria-free Lambs. *Am. J. Hyg.*, 11: 362-370. 1930.
- (5) BECKER, E. R., AND HSIUNG, T. S. The Method by which Ruminants Acquire their Fauna of Infusoria and Remarks Concerning the Host Specificity of These Protozoa. *Proc. Natl. Acad. Sci. in U. S.*, 15: 684-690. 1930.
- (6) BECKER, E. R., AND TALBOTT, M. The Protozoan Fauna of the Rumen and Reticulum of American Cattle. *Iowa State College J. Sci.*, 1: 345-371. 1927.
- (7) COLE, H. H., HUFFMAN, C. F., KLEIBER, M., OLSON, T. M., AND SCHALK, A. F. A Review of Bloat in Ruminants. *J. Animal Sci.*, 4: 183-236. 1945.

- (8) FAIRBANKS, W. B., AND KRIDER, J. L. Factors Involved in the Synthesis of Vitamins in the Ruminant. *North Am. Veterinarian*, 25: 97-100. 1944.
- (9) FERBER, K. E. Die Zahl und Masse der Infusorien im Pansen und ihre Bedeutung für den Eiweissaufbau beim Wiederkäuer. *Z. Tierzücht. Zuchtungsbiol.*, 12, 1: 31-63. 1928.
- (10) FERBER, K. E., UND WINOGRADOWA-FEDOROWA, T. Zählung und Teilungsquote der Infusoria im Pansen der Wiederkäuer. *Biol. Zentr.*, 49: 321-328. 1929.
- (11) GRUBY, D., AND DELAFOND, ONESINE. Recherches sur des animaleules se développant sans l'estomac et dans les intestins pendant la digestion des animaux herbivores et carnivores. *Compt. rend. acad. sci.*, 17: 1305-1308. 1843.
- (12) HALE, E. B., DUNCAN, C. W., AND HUFFMAN, C. F. Rumen Digestion Studies. II. Studies in the Chemistry of Rumen Digestion. *J. Nutrition*, 34: 747-758. 1947.
- (13) HASTINGS, B. G. Significance of the Bacteria and Protozoa of the Rumen of the Bovine. *Bact. Rev.*, 8: 235-254. 1944.
- (14) HUFFMAN, C. F. Searching for Dairy Secrets. *Holstein-Friesian World*, 38, 17: 863-864, 886. 1941.
- (15) HUNGATE, R. E. Further Experiments on Cellulose Digestion by the Protozoa in the Rumen of Cattle. *Biol. Bull.*, 84: 157-163. 1943.
- (16) HUNGATE, R. E. The Symbiotic Utilization of Cellulose. *J. Elisha Mitchell Sci. Soc.*, 62, 1: 9-24. 1946.
- (17) HUNGATE, R. E. Studies on Cellulose Fermentation. III. The Culture and Isolation of Cellulose-decomposing Bacteria from the Rumen of Cattle. *J. Bact.*, 53: 631-645. 1947.
- (18) HUNT, C. H., KICK, C. H., BURROUGHS, E. W., BETHIKE, R. M., SCHALK, A. F., AND GERAUGH, P. Studies on Riboflavin and Thiamine in the Rumen Contents of Cattle. *J. Nutrition*, 25: 207-216. 1943.
- (19) JOHNSON, B. C., HAMILTON, T. S., ROBINSON, W. B., AND GAREY, J. C. On the Mechanism of Non-protein Nitrogen Utilization by Ruminants. *J. Animal Sci.*, 3: 287-298. 1944.
- (20) KUDO, R. R. *Protozoology*. 3rd ed. 778 pp. Charles C. Thomas, Springfield, Ill. 1947.
- (21) LARDINOIS, C. C., MILLS, R. C., ELVEHJEM, C. A., AND HART, E. B. Rumen Synthesis of the Vitamin B Complex as Influenced by Ration Composition. *J. Dairy Sci.*, 27: 579-583. 1944.
- (22) MANGOLD, E. *Handbuch der Ernährung und des Stoffwechsels der landschaftlichen Nutztiere als Grundlagen der Fütterungslehre*. Bd. 2, pp. 156-182. Julius Springer, Berlin. 1929.
- (23) MANGOLD, E. Die Infusoria des Pansens und ihre Bedeutung für die Ernährung der Wiederkäuer. *Biedermann's Zentr. A.*, 3: 161-187. 1933.
- (24) McELROY, L. W., AND GOSS, H. A Quantitative Study of Vitamins in the Rumen Contents of Sheep and Cows Fed Vitamin-low Diets. I. Riboflavin and Vitamin K. *J. Nutrition*, 20: 527-540. 1940. II. Vitamin B₆ (Pyridoxine). *J. Nutrition*, 20: 541-550. 1940. III. Thiamine. *J. Nutrition*, 21: 163-173. 1941. IV. Pantothenic Acid. *J. Nutrition*, 21: 405-409. 1941.
- (25) McELROY, L. W., AND JUKES, T. H. Formation of the Anti-egg White Injury Factor (Biotin) in the Rumen of the Cow. *Proc. Soc. Expt. Biol. Med.*, 45: 296-297. 1940.
- (26) SCHWARZ, C. Die ernährungsphysiologische Bedeutung der Mikroorganismen in den Vormägen der Wiederkäuer. *Biochem. Z.*, 156: 130-137. 1925.
- (27) VAN DER WATH, J. G., AND MYBURGH, S. J. Studies on the Alimentary Tract of Merino Sheep in South Africa. VI. The Role of Infusoria in Ruminant Digestion with some Remarks on Ruminant Bacteria. *Onderstepoort J. Vet. Sci. and Animal Ind.*, 17: nos. 1 and 2. 1941. (Original not seen.)
- (28) WEGNER, M. I., BOOTH, A. N., BOHSTEDT, G., AND HART, E. B. The Utilization of Urea by Ruminants as Influenced by the Level of Protein in the Ration. *J. Dairy Sci.*, 24: 835-844. 1941.

HOMOGENIZED MILK VII. EFFECT OF AGITATION DURING FREEZING ON THE KEEPING QUALITY OF FROZEN HOMOGENIZED MILK

C. J. BABCOCK,¹ ERNEST WINDHAM, AND COLONEL RAYMOND RANDALL, VC.
*Veterinary Division, Army Medical Department, Research and Graduate School,
Army Medical Center, Washington 12, D. C.*

Previous studies (5, 6) have proved that homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration for longer periods of time than usually are necessary before use. Further, it has been found that homogenized milk of good quality may be kept as long as 120 hours at 1.67° C. before freezing without adversely affecting the keeping quality of the frozen product. A more recent study (7) has shown that the addition of sodium citrate with ascorbic acid increases the time that homogenized milk can be stored in the frozen state without protein flocculation or flavor deterioration.

Reports on the use of agitation during the freezing of homogenized milk have not been found. However, experimental work reported by Doan and Baldwin (9) indicates that the destruction of the fat emulsion in milk and cream frozen without agitation is caused by internal pressures developed in the congealing mass. This is a result of initial surface freezing followed by the expansion of water in the body of the product as it is converted to ice. Later, Doan and Leeder (10) reported that the internal pressure was greatly reduced, if not eliminated, and the fat emulsion of the product little affected when concentrated milk was partially frozen in an ice cream freezer with agitation and then filled into small containers for final freezing and storage. Civfl (8) analyzed the outer layer, the part which froze first, as well as the top, middle and bottom portions of the remainder of the sample and found that the central portion was richer in fat, casein, albumin globular sugar and chloride ion than the upper or lower portions. The outer layer was found to be the poorest in these constituents. Babcock *et al.* (3, 4) and Trout (13) have shown that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample. Other studies (2, 11, 12, 14) have also shown that freezing and storage temperatures affect the physical character of homogenized milk.

The present experiments were undertaken to determine the effect of agitation during freezing on the keeping quality of frozen homogenized milk.

EXPERIMENTAL

Homogenized milk with a fat content of 3.8 per cent pasteurized by holding at 155° F. for 30 minutes and packaged in paper containers by a commercial dairy was used. Quart samples were used to determine the efficiency of the agitation as shown by milk solids distribution. One-half pint samples were used to determine keeping quality as shown by protein flocculation and flavor.

Received for publication May 14, 1949.

¹ Formerly Lt. Colonel, SnC., AUS. Present address: Dairy Branch, Production Marketing Administration, U. S. Department of Agriculture.

The samples were frozen and stored at about $-17.8^{\circ}\text{C}.$ ² One half of the samples were placed in the freezer and kept in a stationary condition during freezing. The other half of the samples were frozen in the same freezer by placing them in a box attached to a wheel which was revolved by a motor at the rate of 35 r.p.m. (figure 1). This group of samples then was rotated for 72 hours to insure that they would be frozen solidly before they were removed from the rotator. All but three of the quart samples were removed from the freezer shortly after freezing and divided, while in a frozen condition, into top, middle and bottom portions. Each part was thawed and analyzed for fat, protein, total solids



FIG. 1. Rotator used for agitating homogenized milk during freezing.

and ash content to determine the distribution of the milk solids. In addition to the chemical analyses, the milk solids distribution was verified by determining the freezing point of the respective portions of the thawed milk. Chemical analyses were made by methods described in a previous report (3). The freezing point was determined by using a Hortvet cryoscope in accordance with AOAC methods (1). The three quart samples were removed from the rotator after freezing and left in the freezer in a stationary upright position. They were removed after 22, 39 and 82 days and divided while frozen into two equal portions. Each portion was analyzed for total solids, fat, and protein. The degree of separation in each portion also was determined. The half-pint samples were removed and thawed for examination at intervals of about 10 days, beginning with

² The thermostat controlling the temperature of the freezer had a sufficient lag to cause a maximum temperature variation of about $8^{\circ}\text{C}.$

the 56th day of storage. The degree of separation of the milk was measured by determining the amount of sediment in 50 ml. quantities by centrifuging as was done in previously reported experiments (2). Flavor determinations were made by a panel of three men experienced in milk judging.

The effect of rotating during freezing on the distribution of solids in frozen homogenized milk is shown in table 1. When quart samples of homogenized milk

TABLE 1

Effect of rotating during freezing on the distribution of solids in frozen homogenized milk as shown by chemical analysis and freezing point

Sample number	Method of handling	Section of sample	Freezing point after thawing	Fat	Protein	Total solids	Ash
			(° C.)	(%)	(%)	(%)	(%)
1	Stationary	Top	-.397	2.84	2.54	9.77	0.58
		Middle	-.511	3.09	3.06	11.64	0.71
		Bottom	-.657	3.97	3.85	14.68	0.86
2	Stationary	Top	-.389	2.80	2.52	9.55	0.55
		Middle	-.499	3.18	2.97	11.60	0.67
		Bottom	-.674	3.69	3.95	14.93	0.85
3	Rotated	Top	-.500	3.36	3.11	11.75	0.70
		Middle	-.527	3.36	3.23	12.24	0.71
		Bottom	-.532	3.47	3.23	12.36	0.75
4	Rotated	Top	-.474	3.20	2.91	11.22	0.65
		Middle	-.537	3.52	3.24	12.36	0.73
		Bottom	-.539	3.40	3.25	12.38	0.73
5	Stationary	Top	-.383	2.82	2.44	9.32	0.55
		Middle	-.499	3.36	3.02	11.55	0.69
		Bottom	-.687	4.42	4.02	15.38	0.90
6	Rotated	Top	-.525	3.65	3.22	12.23	0.72
		Middle	-.583	3.99	3.50	13.39	0.79
		Bottom	-.422	2.99	2.64	10.09	0.61
7	Rotated	Top	-.442	3.10	2.68	10.42	0.61
		Middle	-.531	3.67	3.11	12.29	0.73
		Bottom	-.590	3.87	3.49	13.33	0.79
8	Rotated	Top	-.524	3.58	3.21	12.23	0.73
		Middle	-.540	3.65	3.14	12.21	0.73
		Bottom	-.526	3.63	3.15	12.16	0.73
9	Rotated	Top	-.506	3.54	3.12	11.81	0.68
		Middle	-.552	3.78	3.28	12.60	0.76
		Bottom	-.520	3.60	3.15	11.96	0.71

were rotated during freezing, the concentration of milk solids was practically the same in the top, middle and bottom sections. The control samples, which were frozen in a stationary position, gave results similar to those previously reported (3, 4) in that there was a tendency for the solids to settle toward the bottom. There was a greater concentration in the bottom third than in the middle third and a greater concentration in the middle third than in the top third. When the quart samples were rotated during freezing, the freezing point of the top, middle and bottom sections of the milk after thawing was practically the same. In those samples that were not rotated during freezing, the settling of the solids was reflected in the freezing point of the different sections of the milk. In each case the freezing point of the middle section was lower than the freezing point of

the top section and the freezing point of the bottom section was lower than that of the middle section.

The effect of rotating during freezing on the distribution of solids in frozen homogenized milk after storage is shown in table 2. When homogenized milk

TABLE 2

Effect of rotating during freezing on the distribution of solids in frozen homogenized milk after storage

Days in storage	Total solids		Fat		Protein		Sediment in 50 ml.	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
	(%)	(%)	(%)	(%)	(%)	(%)	(ml.)	(ml.)
22	12.55	12.37	3.73	3.68	3.25	3.22	0.02	0.02
39	12.07	12.16	3.62	3.61	3.15	3.16	0.02	0.03
82	12.28	12.17	3.64	3.61	3.21	3.14	0.02	0.03

was rotated during freezing and then stored in a stationary upright position the milk solids remained evenly distributed throughout the milk for 82 days. This further confirms the results previously reported (4) that when homogenized milk was frozen, the concentration of the milk solids in the lower portion of the sample took place during the freezing process and apparently there was no further movement of these solids after the milk was frozen. The degree of separation was similar in the top and bottom sections of the milk.

Having shown that rotating while freezing prevented a concentration of the milk solids in the bottom of the quart container, the half-pint samples were used to determine the effect of rotation during freezing on the keeping qualities as indicated by flavor and the degree of separation. These results are shown in table 3. Agitation during freezing did not improve the keeping quality of

TABLE 3

Effect of rotation during freezing on the keeping quality of frozen homogenized milk

Days in storage	Method of handling	Sediment (ml./50 ml.)	Flavor
56	Stationary	0.03	
70	Stationary	0.10	
77	Stationary	0.30	
85	Stationary	0.5	Oxidized
85	Rotated	0.9	Oxidized
92	Stationary	0.06	Oxidized, stale
92	Rotated	0.03	Oxidized, stale
97	Stationary	0.10	Sl. Oxidized, stale
97	Rotated	0.35	Oxidized, stale
103	Stationary	1.4	Oxidized
103	Rotated	1.2	Oxidized
112	Stationary	1.4	Oxidized, stale
112	Rotated	1.5	Oxidized, stale
118	Stationary	1.6	Oxidized
118	Rotated	1.6	Oxidized

homogenized milk. Furthermore, separation, as shown by the amount of sediment in 50 ml. portions, was not delayed by rotating the milk while freezing.

CONCLUSIONS

When homogenized milk was agitated by rotating at 35 r.p.m. during freezing the chemical analysis and freezing point of various sections of the sample showed that the milk solids remained evenly distributed throughout the sample.

Preventing the concentration of milk solids in the lower portion of homogenized milk by rotating it during freezing does not improve its keeping quality.

Acknowledgement is extended to Elmina Dickson and Edith Giltner for assistance with the analyses.

REFERENCES

- (1) Association of Official Agricultural Chemists, Washington, D. C. *Official and Tentative Methods of Analysis*. 6th Ed. Pp. 312-316. 1945.
- (2) BARCOCK, C. J., ROERIG, R. N., STABILE, J. N., DUNLAP, W. A., AND RANDALL, R. Frozen Homogenized Milk. I. Effects of Freezing and Storage Temperatures on the Physical Characteristics of Homogenized Milk. *J. Dairy Sci.*, 29: 699-706. 1946.
- (3) BARCOCK, C. J., ROERIG, R. N., STABILE, J. N., DUNLAP, W. A., AND RANDALL, R. Frozen Homogenized Milk. II. Effect of Freezing and Storage Temperatures on the Chemical and Bacteriological Properties of Homogenized Milk. *J. Dairy Sci.*, 30: 49-54. 1947.
- (4) BARCOCK, C. J., STABILE, J. N., RANDALL, R., AND WINDHAM, E. S. Frozen Homogenized Milk. III. Stability of Milk Solids Distribution in Frozen Homogenized Milk. *J. Dairy Sci.*, 30: 733-736. 1947.
- (5) BARCOCK, C. J., STABILE, J. N., RANDALL, R., AND WINDHAM, E. S. Frozen Homogenized Milk. IV. Keeping Quality of Frozen Homogenized Milk After Thawing. *J. Dairy Sci.*, 31: 805-810. 1948.
- (6) BARCOCK, C. J., STABILE, J. N., WINDHAM, E. S., EVANS, L. B., AND RANDALL, R. Frozen Homogenized Milk. V. Effect of Age before Freezing on the Keeping Quality of Frozen Homogenized Milk. *J. Dairy Sci.*, 31: 811-815. 1948.
- (7) BARCOCK, C. J., STABILE, J. N., WINDHAM, E. S., AND RANDALL, R. Frozen Homogenized Milk. VI. The Use of Stabilizers in Frozen Homogenized Milk. *J. Dairy Sci.*, 32: 175-182. 1949.
- (8) CIVTL, J. Einfluss des Gefrierens auf Einige Bestandteile und Eigenschaften der Milch. *Milchw. Forsch.*, 12: 409-432. 1931.
- (9) DOAN, F. J., AND BALDWIN, F. B. Observations on the Freezing of Milk and Cream. II. The Destruction of the Fat Emulsion in Frozen Cream. *J. Dairy Sci.*, 19: 225-233. 1936.
- (10) DOAN, F. J., AND LEEDER, J. G. Milk can be Frozen for Sale to Consumers. *Food Inds.*, 16: 532-534, 579. 1944.
- (11) ROADHOUSE, C. L., AND HENDERSON, J. L. How Quick Freezing Affects Keeping Quality of Milk. *Food Inds.*, 12: 54-55. 1940.
- (12) THURSTON, L. M., BROWN, W. C., AND DUSTMAN, R. B. Oxidized Flavor in Milk. *J. Dairy Sci.*, 19: 671-681. 1936.
- (13) TROUT, G. M. The Freezing and Thawing of Milk Homogenized at Various Pressures. *J. Dairy Sci.*, 24: 277-287. 1941.
- (14) WEBB, B. H., AND HALL, S. A. Some Physical Effects of Freezing upon Milk and Cream. *J. Dairy Sci.*, 18: 275-287. 1935.

THE EFFECT OF THE ADMINISTRATION OF VARIOUS FATTY ACIDS ON THE BLOOD KETONE LEVELS OF RUMINANTS¹

L. H. SCHULTZ AND VEARL R. SMITH

Department of Dairy Husbandry

and

H. A. LARDY

Department of Biochemistry, University of Wisconsin, Madison

The widespread occurrence of ketosis in dairy cattle has made this a disease of practical as well as of theoretical importance. Considerable work has been done *in vitro* on the precursors of the ketone bodies, and some work has been done *in vivo* with laboratory animals, but very little has been done with ruminants. Since ketosis is a much greater practical problem in ruminants than in any other species, and since ruminants very often do not react in the same manner as smaller laboratory animals, it was deemed desirable to study this problem using ruminants as experimental animals. A preliminary report of this work appeared earlier in abstract form (12).

REVIEW OF LITERATURE

Jowett and Quastel (6) incubated normal saturated fatty acids containing two to ten carbon atoms in the presence of liver slices. They concluded that the four, six and eight carbon compounds produced acetoacetic acid most readily, that the ten carbon acid was slightly less active and that acetic acid was considerably less active. Propionic acid did not form acetoacetic acid, and the other odd-numbered acids produced very small amounts. Medes *et al.* (10) showed that ketone bodies arise as intermediates of acetate oxidation in animal tissues *in vitro*. Lehninger (7) has succeeded in obtaining liver suspensions which under the proper conditions readily oxidize all of the normal saturated fatty acids containing four to eighteen carbon atoms to yield acetoacetic acid as end product.

MacKay *et al.* (8) fed acetic acid to a phlorrhizinized dog and to fasting rats and demonstrated an increased production of ketone bodies. Swendseid *et al.* (13), using the heavy isotope of carbon, C¹³, showed that acetic acid takes part in the synthesis of the acetone bodies in the fasting rat. Forbes (5) administered acetic acid to one goat and observed no increase in ketone body excretion. MacKay *et al.* (9) fed normal, saturated fatty acids with four, six, eight, and ten carbon atoms to rats and showed that all were ketogenic, even when adequate amounts of carbohydrate were fed.

Received for publication June 1, 1949.

¹ These data were taken from a thesis submitted by L. H. Schultz to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

More recently Phillipson (11) demonstrated that the lower fatty acids were important products of bacterial fermentation in the rumen, and that the acids produced were primarily acetic, propionic and butyric, with acetic predominating. These acids were shown to be absorbed directly into the blood stream from the rumen, reticulum and omasum. Folley and French (4) have shown that ruminant tissues apparently have a greater ability to metabolize acetate than do those from non-ruminants.

EXPERIMENTAL PROCEDURE

Various fatty acids were administered, either orally or intravenously, to goats and the blood ketone levels were followed. The animals used were four mature female goats which averaged 98 lb. in weight and had been in lactation about 5 months. Except for one test where they were fasted, the goats were fed and handled normally. The goats were used at random in the course of the experiments. However, the fatty acids which gave positive results all were tested at one dosage level on the same goat to check the differences obtained in blood ketone levels. One study on acetic acid administration was made with a phlorhizinized goat. The goat used for this experiment was a young male castrate goat weighing 60 lb. The animal was fed normally. One g. of phlorhizin dissolved in 3 ml. of propylene glycol was administered for 3 days prior to the study. The animal was placed in a metabolism cage and complete daily urine collections were made.

The fatty acids used were acetic, propionic, butyric, caproic, caprylic, capric and oleic. Other organic acids tested were lactic and succinic. Corn oil also was used. For oral administration, the liquid fatty acids and the organic acids were diluted with 400 ml. of water and introduced into the rumen by means of a stomach tube. Solid fatty acids and corn oil were administered by capsule. Fifteen g. dosages were used first and then all of the experiments were repeated at least once using 30 g. dosages. For intravenous injections only acetic and butyric acids were used, and they were diluted with 250 ml. of physiological saline and injected into the jugular vein. A single 10 g. dosage was used for acetic and two dosages, one of 5 g. and one of 10 g., were used for butyric acid in the injection studies.

Blood samples were taken immediately prior to the administration of substances to be tested, and usually at 0.25, 0.5, 1, 1.5, 2, 3 and 4 hr. intervals afterward. Normal values were determined for a 3-day period prior to the start of this study. They ranged from 1.1 to 4.0 mg. per cent total ketone bodies.

Blood ketone determinations were carried out on a Folin-Wu (3) blood filtrate according to the method of Behre and Benedict (1). The colorimetric determination of acetone in the distillate was carried out according to the method of Block and Bolling (2), using a Coleman spectrophotometer. All figures given represent total ketone bodies expressed as acetone.

RESULTS AND DISCUSSION

Contrary to the results obtained by other workers with tissue slices and with intact rats and dogs, acetic acid showed no definite ketogenic activity when administered orally to goats, as determined by following blood ketone levels. It is recognized, however, that our experiments were set up somewhat differently

TABLE 1

Total blood ketone levels following oral administration of acetic acid to normal, fasted and phlorhizinized goats

Time after administration	Normal		Fasted		Phlorhizinized
	Amt. administered		Amt. administered		Amt. administered
	15 g.	30 g.	15 g.	30 g.	30 g.
	Total blood ketones		Total blood ketones		Total blood ketones
(Hr.)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)
0	1.5	1.3	1.9	1.2	2.7
0.25	1.9	1.5	2.9	0.9	3.0
0.5	1.5	3.4	1.1	0.8	2.8
1	2.2	3.9	1.2	1.0	2.1
1.5	2.5	3.8			
2	4.0	3.3	1.8	1.1	2.9
3	1.6	1.4	1.8	1.7	3.9
4	1.9	1.2	2.3	1.8	4.1

than those in which rats and dogs were used. As shown in table 1, acetic acid was given in 15 and 30 g. doses to goats fed normally and to goats which had been fasted for 36 hr. No significant changes were noted in blood ketone levels in any

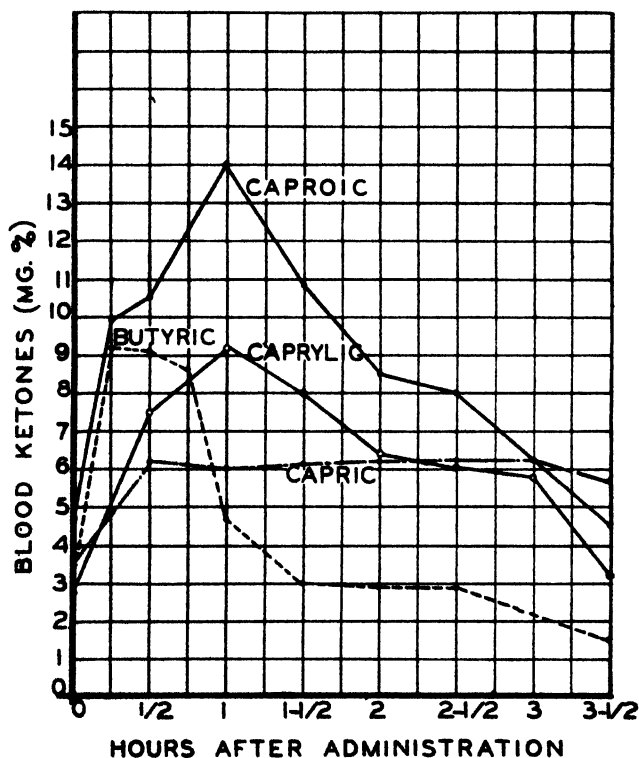


FIG. 1. The effect of oral administration of 30 g. of various fatty acids on the blood ketone level.

of the trials. The goat which had been treated with phlorhizin was excreting 35 to 40 g. of sugar daily, but the blood sugar values were within the normal limits. Again no significant changes were noted in blood ketones following acetic acid administration, as shown in table 1.

The fatty acids containing four, six, eight and ten carbon atoms caused definite increases in blood ketones following administration. Four trials were made on butyric acid and at least two on each of the other acids. Figure 1

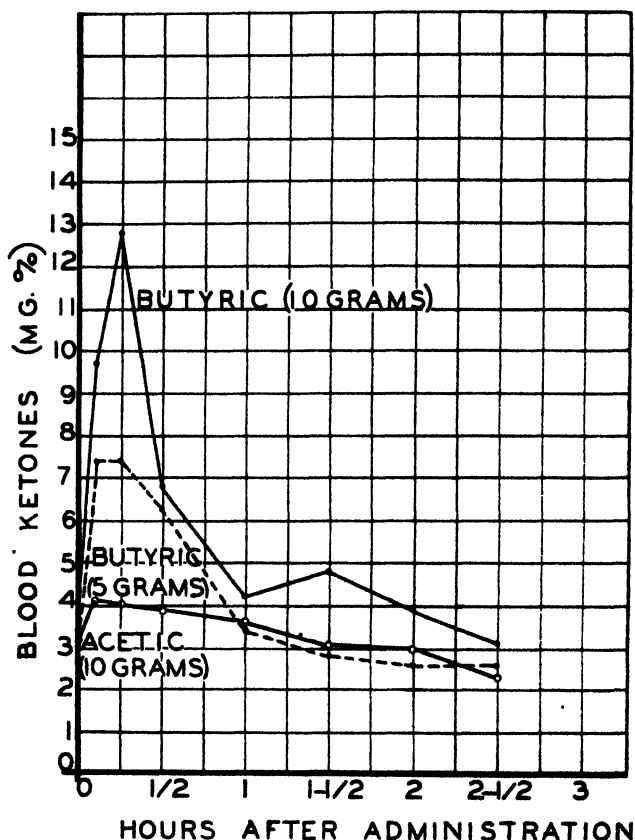


FIG. 2. The effect of intravenous injection of acetic and butyric acids on the blood ketone level.

shows the results of the 30 g. dosage level when each of the four acids was administered to goat no. 45. Butyric caused the most rapid increase in blood ketones, the peak being reached in 0.5 hr., and the values returned to normal more rapidly than in the case of the other acids. Peaks of blood ketones were reached in about 1 hr. for the other acids. Caproic acid caused the greatest rise in blood ketones and capric the least, with butyric intermediate. This was the general observation in all of the tests, although there were some trials where butyric caused a greater increase than caproic. In general, the longer the acid chain

the longer it took for the blood values to return to normal. In most cases the values were back to normal in 3 to 4 hr.

Essentially the same results were obtained by intravenous injection of acetic and butyric acids as with oral administration, indicating that the rumen micro-organisms were not involved in the results obtained from oral administration. As shown in figure 2, 10 g. of acetic acid injected intravenously caused no change in blood ketone levels beyond the normal variations. Butyric acid, on the other hand, in both 5 and 10 g. dosage levels, caused definite and rapid increases in blood ketones, maximum values being reached in 15 min. Smaller amounts were needed intravenously than orally to give comparable increases in blood ketones.

Several other fatty or organic acids, as well as corn oil, were tested for ketogenic activity with essentially negative results. Table 2 shows the total blood

TABLE 2

Total blood ketone levels following oral administration of miscellaneous acids and corn oil

Time after administration	Substances administered (30 g.)					
	Propionic acid	Lauric acid	Oleic acid	Lactic acid	Succinic acid	Corn oil
	Total blood ketones					
(Hr.)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)	(mg. %)
0	3.3	2.4	4.4	2.2	1.1	2.8
0.5	1.0	2.4	4.0	3.3	1.1	2.7
1	1.8	2.8	3.2	2.1	1.0	3.0
2	1.4	3.4	3.2	1.0	0.8	2.5
3	1.7	3.3	2.0	1.3	0.8	2.4
4	1.8	4.4	1.2	1.6	0.8	2.7

ketone levels following oral administration of 30 g. dosages of six different substances. Propionic acid caused no perceptible increase, thus confirming work with tissue slices and laboratory animals. Lauric acid gave negative results under the conditions of this study. Oleic acid failed to cause any increases in blood ketones. Lactic and succinic acids, as well as corn oil, also showed negative results.

SUMMARY

The oral administration of acetic acid is fasted, non-fasted or phlorhizinized goats did not result in an increase in blood ketones. Butyric, caproic, caprylic and capric acids administered orally caused increases in blood ketones of 5 to 10 mg. per cent. Maximum levels of ketones were usually reached in 15 min. for butyric acid and in 1 hr. for the other three acids. The greatest increases were observed with butyric and caproic acids. Values returned to normal in about 3 hr. Propionic, lauric, oleic, lactic or succinic acids, administered in equivalent amounts, caused no significant rise in blood ketones. Corn oil also gave negative results.

Intravenous injection of acetic or butyric acids gave results similar to oral administration. Acetic acid caused no significant changes, while butyric acid

caused rapid increases in blood ketones. Less acid was needed intravenously than orally to give comparable increases in blood ketones.

REFERENCES

- (1) BEHR, J. A., AND BENEDICT, S. R. A Colorimetric Method for the Determination of Acetone Bodies in Blood and Urine. *J. Biol. Chem.*, **70**: 487-494. 1926.
- (2) BLOCK, R. J., AND BOLLING, D. *The Determination of the Amino Acids*, Rev. Ed. Burgess Publishing Co., Minneapolis. 1940.
- (3) FOLIN, O., AND WU, H. A System of Blood Analysis. *J. Biol. Chem.*, **38**: 81-110. 1919.
- (4) FOLLEY, S. J., AND FRENCH, T. H. Utilization of Acetate by Tissues of the Ruminant. *Biochem. J.*, **43**: Proc. lv. 1948.
- (5) FORBES, R. M. Ketosis in Ruminants. *Cornell Vet.*, **33**: 27-47. 1943.
- (6) JOWETT, M., AND QUASTEL, J. H. Studies in Fat Metabolism. II. The Oxidation of Normal Saturated Fatty Acids in the Presence of Liver Slices. *Biochem. J.*, **29**: 2159-2180. 1935.
- (7) LEHNINGER, A. L. On the Activation of Fatty Acid Oxidation. *J. Biol. Chem.*, **161**: 437-451. 1945.
- (8) MACKAY, E. M., BARNES, R. H., CARNE, H. O. AND WICK, A. M. Ketogenic Activity of Acetic Acid. *J. Biol. Chem.*, **135**: 157-163. 1940.
- (9) MACKAY, E. M., WICK, A. N., AND BARNUM, C. P. Ketogenic Action of Short Chain, Even Numbered Carbon Fatty Acids in Carbohydrate-Fed Animals. *J. Biol. Chem.*, **135**: 183-187. 1940.
- (10) MEDES, G., FLOYD, N. F., AND WEINHOUSE, S. Fatty Acid Metabolism. III. Ketone Bodies as Intermediates of Acetate Oxidation in Animal Tissues. *J. Biol. Chem.*, **162**: 1-9. 1946.
- (11) PHILLIPSON, A. T. The Role of the Microflora of the Alimentary Tract of Herbivora with Special Reference to Ruminants. III. Fermentation in the Alimentary Tract and the Metabolism of the Derived Fatty Acids. *Nutrition Abstracts and Rev.*, **17**: 12-18. 1947.
- (12) SCHULTZ, L. H., SMITH, V. R., AND LARDY, H. A. The Effect of the Administration of Various Fatty Acids on the Blood Ketone Levels of Ruminants. *J. Animal Sci.*, **7**: 544. 1948.
- (13) SWENDSEID, M. E., BARNES, R. H., HEMINGWAY, A., AND NIER, A. O. The Formation of Acetone Bodies from Acetic Acid. *J. Biol. Chem.*, **142**: 47-51. 1942.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

OCTOBER, 1949

NUMBER 10

THE COMPARATIVE VALUE OF CHOCOLATE AND WHOLE MILK AS A SOURCE OF RIBOFLAVIN FOR THE RAT

C. W. GEHRKE,¹ L. C. THOMAS, M. R. SHETLAR,² AND T. CHITWOOD³

Department of Biology and Chemistry, Missouri Valley College, Marshall, Missouri

Several recent investigations have shown that the photochemical destruction of riboflavin in milk is quite rapid. Peterson *et al.* (1) found losses of riboflavin, as detected by the fluorometric procedure, in pint glass bottles of whole milk exposed to direct sunlight of 28, 50, 66 and 72 per cent after 30, 90, 120 and 210 minutes of exposure, respectively. Similar reports were given by Ziegler (4). Shetlar *et al.* (2) reported that chocolate milks lose riboflavin very slowly when exposed to sunlight, the average loss of riboflavin after 4 hours for five different brands being about 12 per cent as compared to a loss of 80 per cent for whole milk. Warner and Sutton (3) recently reported that calves fed on a photolyzed whole milk diet developed characteristic riboflavin deficiency symptoms. The reports by the above authors indicate the importance of preventing the exposure of milk to sunlight with the resultant inactivation of part of the riboflavin. Loss of riboflavin in whole milk due to light exposure easily could result in at least a partial riboflavin deficiency. Chocolate milk as manufactured compares favorably to whole milk in riboflavin content as determined by the fluorometric procedure developed by Shetlar *et al.* (2). The much slower photochemical loss of riboflavin in chocolate milk perhaps is due to the extra protection afforded by the added chocolate. This would indicate that chocolate milk might prove advantageous if the milk during delivery was subjected to much exposure to light, provided that the chocolate does not interfere with the assimilation of riboflavin after consumption.

The common substitution of chocolate milk for whole milk in the diet, especially of children, has been of concern to nutritionists until it could be established that no vital deficiency resulted from the substitution. Consequently, it seemed desirable to check biologically the comparative value of chocolate milk and whole milk as a source of riboflavin.

EXPERIMENTAL METHODS

The white rats used were selected within an inbred colony descended from a single pair after 3 years of inbreeding. Both males and females were used and

Received for publication March 16, 1949.

¹ Now at the University of Missouri, Columbia.

² Now at the University of Oklahoma, Oklahoma City.

³ Now at Raytown High School, Raytown, Mo.

all were put on experiment at the time of weaning, when weights varied from 48 to 52 g. and average weight of the 22 animals was 48.9 g.

The animals were given a basal diet⁴ of the following percentage composition: sucrose, 68; vitamin-test casein, 18; vegetable oil, 10; U.S.P. salt mixture no. 2, 4. The basal diet was a vitamin B-complex test diet nutritionally complete but free of the vitamin B-complex and vitamins A and D. The daily vitamin supplement of 20 γ thiamine, 20 γ pyridoxine, 6 mg. choline chloride, and 200 γ calcium pantothenate was dissolved in 1 ml. of a 25 per cent ethanol solution; this was pipetted into small dishes and given separate from the ration. As a source of vitamins A and D, three drops of halibut liver oil were given once weekly to each animal. One ml. of a 25 per cent ethanol solution containing 20 γ of riboflavin was given daily to the positive control group.

The experimental animals were given 10 ml. of whole or chocolate milk daily as a riboflavin supplement. This compares favorably with the amount of riboflavin given to the positive control group, since whole and chocolate milk contain approximately 2 γ of riboflavin per ml. The animals were housed in individual wire cages having 0.5-inch mesh screen bottoms.

In series 1, six rats which served as the negative control group were fed a complete basal diet except for the absence of riboflavin. This series was depleted of riboflavin for 42 days. The period of depletion was determined by the characteristic symptoms of riboflavin deficiency, such as muscular incoordination, keratitis and alopecia. At the end of the depletion period this series was divided into lots 1 and 2, each containing three rats. Lot 1 was given 10 ml. of whole milk daily as a supplement for 23 more days. Lot 2 was given 10 ml. of chocolate milk daily as a supplement for the same period. Recovery from their characteristic symptoms of riboflavin deficiency would indicate the supplement to be a satisfactory source of riboflavin.

In series 2, six rats, constituting the positive control group, were fed the same basal diet as series 1 except that the diet was supplemented with 20 γ of riboflavin daily. Two of these rats died early in the experiment.

In series 3, six rats were fed the same basal diet as lot 1, except that 10 ml. of whole milk was given daily as a supplement from the beginning of the experiment.

In series 4, six rats were fed the same basal diet as lot 2, except that 10 ml. of chocolate milk was given daily as a supplement from the beginning of the experiment.

The absence of any evidence of riboflavin deficiency in either series 3 or 4 would indicate that the supplements were a satisfactory source of riboflavin.

The rats were weighed three times a week and the average weight of the rats in each series was calculated. Parallel records for each series were made for 39 days, after which series 2, 3 and 4 were taken out of the experiment and series 1, as lots 1 and 2, was continued on experiment for 23 more days. The effect on growth was used as the criterion for the measurement of the availability and assimilation of riboflavin by the rats.

⁴ General Biochemicals, Inc., Chagrin Falls, Ohio.

RESULTS

The data from this series of experiments are expressed graphically in fig. 1. The data show that when the rats were weaned, unless milk was given immediately along with the solid food, they lost weight for a few days until they learned to take the solid food. Therefore, each growth curve has to be adjusted for this loss by calculating the rate of growth over the period during which there was a continued increase in weight.

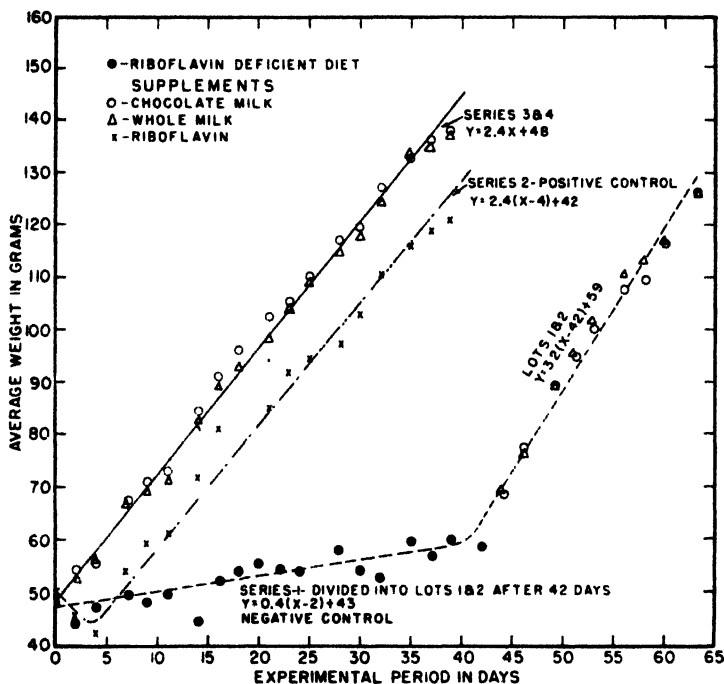


FIG. 1. The value of chocolate and whole milk as a source of riboflavin in the diet of the rat.

While the rate of growth is not a constant but decreases slightly as the growth approaches the limit of maturity, if the sectors covered in the experiment are not too great, a straight line will approximate the curve using the average daily gain as the rate. Using the positive control group, series 2, to obtain the normal growth curve, the equation $y = 2.4(x - 4) + 42$ is obtained, where 2.4 is the average daily gain in g., x is the age of the rat in days, $x - 4$ adjusts for the initial loss of weight for the ages recorded in days from which the experiment began, 42 is the initial weight and y is the weight in g. of the rat at age $x - 4$ in days.

On the basis of the above equation the expected curves for experimental series 3 and 4 can be plotted, provided the necessary riboflavin is in the supplements. A deviation from this expected curve would indicate deficiency of this vitamin.

This equation is: $y = 2.4x + 48$. Superimposing the actual curves on this by plotting the observed data, using triangles for the whole milk, series 3, and circles for the chocolate milk, series 4, both series show a very good fit to the expected curve. The results show that chocolate milk, as well as whole milk, is an adequate supplement as a source of riboflavin in the diet of the rat at the levels investigated. Possibly some difference might have been found at a riboflavin level too low to support optimum growth; however, this possibility was not investigated in this work.

The negative group, series 1, made very little gain during the 42 days they were on the deficient diet and developed symptoms of vitamin deficiency, such as sore eyes and rough coat, indicating a severe deficiency of riboflavin. The average daily gain was only 0.4 g. per day. This period of their life can be represented by the curve: $y = 0.4(x - 2) + 43$. A remarkable change took place when chocolate or whole milk was added to their diet at the end of 42 days. They immediately began to gain at an accelerated growth rate that tended to compensate for the retarded condition. Using the same symbols for the whole milk lot 1 and the chocolate milk lot 2 as before, the average weights coincide a surprising number of times. Both curves fit very closely to that expressed by the equation: $y = 3.2(x - 42) + 59$.

SUMMARY AND CONCLUSIONS

The comparative value of chocolate and whole milk as a source of riboflavin for the rat was investigated. Rats fed chocolate milk had the same rate of growth as rats fed whole milk when both milks were added to a basal diet deficient only in riboflavin. The rate of growth of rats receiving the basal diet supplemented with either milk also was the same as that of rats receiving supplements of crystalline riboflavin. Therefore, at the level of feeding used in this experiment, chocolate milk contains enough biologically available riboflavin to insure maximum growth of the rat. No evidence was found to indicate that the added chocolate interferes with the biological availability of riboflavin.

ACKNOWLEDGMENT

The authors are grateful to the Tullis-Hall Dairy of Sedalia, Mo., for supplying the chocolate and whole milk used in this investigation.

REFERENCES

- (1) PETERSON, W. J., HAIG, F. M., AND SHAW, A. O. Destruction of Riboflavin in Milk by Sunlight. *J. Am. Chem. Soc.*, **66**: 662-663. 1944.
- (2) SHETLAR, M. R., SHETLAR, C. L., AND LYMAN, L. F. Determination of Riboflavin in Chocolate Milk and the Comparative Photochemical Losses of Riboflavin in Chocolate and Whole Milk. *J. Dairy Sci.*, **28**: 873-878. 1945.
- (3) WARNER, R. G., AND SUTTON, T. S. Performance of Calves on a Photolyzed Milk Diet. *J. Dairy Sci.*, **31**: 675-676. 1948.
- (4) ZIEGLER, J. A. Photochemical Destruction of Vitamin B₂ in Milk. *J. Am. Chem. Soc.*, **66**: 1039-1040. 1944.

THE INSTABILITY OF ASCORBIC ACID IN WATER, WITH ADDED COPPER OR HYDROGEN PEROXIDE OR BOTH¹

R. W. BELL AND T. J. MUCHA

*Division of Dairy Products Research Laboratories, Bureau of Dairy Industry,
Agricultural Research Administration, U. S. Department of Agriculture,
Washington 25, D. C.*

Results obtained in our laboratories caused us to suspect that impurities in the water from a laboratory still were hastening the oxidation of ascorbic acid solutions. They led us to investigate the effect of different factors, particularly the source of the water, upon the stability of ascorbic acid solutions. A water solution of ascorbic acid is used in standardizing the dye, 2,6-dichlorophenol-indophenol, with which the amount of this form of vitamin C in milk and in other fluids can be determined.

Water from the laboratory still was obtained by evaporating tap water in a reservoir indirectly heated by steam, collecting the condensate in large earthenware jars, and then drawing it from a tin lined bronze faucet through 12 to 14 feet of tin lined iron pipe. However, the condensate came in contact with brass couplings which contain a high proportion of copper.

The concentration of ascorbic acid in the various water solutions and in the milk was determined by titration in acid solution (sulfuric) with 2,6-dichlorophenolindophenol (4, 14).

In figure 1 are shown the rates of oxidation of ascorbic acid at 30° C. in (a) water distilled in pyrex glassware, (b) water from the laboratory still, and (c) water from the laboratory hot water tap. The amounts of ascorbic acid in these waters, immediately after its addition, were 19.9, 20.0 and 16.5 mg./l., respectively. Also shown in this figure are the rates of oxidation of ascorbic acid dissolved in glassware-distilled water in approximately the same concentration with 0.15 and 0.5 p.p.m. of copper added. The crystalline ascorbic acid was dissolved in a small volume of glassware-distilled water; the copper, in the form of copper sulphate, was added in a standardized solution. These solutions were added to glassware-distilled water at 30° C., made up to the desired volume and mixed by pouring from one flask to another.

The rate of oxidation of ascorbic acid in the tap water was faster than in the glassware-distilled water to which 0.5 p.p.m. of copper had been added. When as little as 0.15 p.p.m. of copper was added to the glassware-distilled water, 83 per cent of the ascorbic acid was oxidized within 60 minutes.

The acid intensity (pH) and oxidation-reduction potential (E_h) of a solution influence the rate of oxidation of ascorbic acid. The former was shown by Barron, DeMeio and Klemperer (2), the latter by Kenny (8). Other things being equal, the rate of oxidation of ascorbic acid is faster in an alkaline than it is in an acid solution. Of two ascorbic acid solutions that are similar except

Received for publication May 23, 1949

¹ This work was done with funds from the Research and Marketing Act of 1946.

that one has a higher E_h than the other, the ascorbic acid in the former will be less stable than in the latter.

Repeated pH and E_h measurements of water from the glassware still, the laboratory still and the hot water tap gave surprisingly constant values, even though the waters were poorly buffered and poised. On the average, the addition of 20 mg. of ascorbic acid to a liter of tap water lowered the pH to 7.5 from 7.7. The E_h was then +0.41. When the glassware-distilled and laboratory still waters were similarly acidulated their pII values decreased nearly 2 units or from about 6.6 to 4.6. However, their E_h values were +0.41 and +0.34, respectively. Because of the lack of buffering and poisoning of these solutions, these values must be considered only relative rather than absolute.

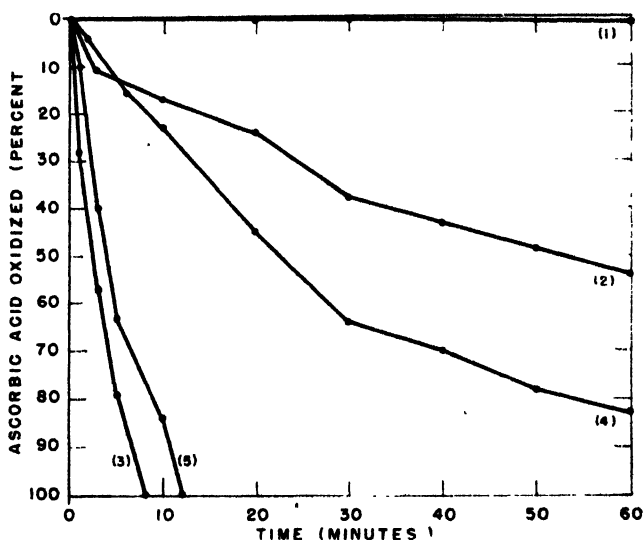


FIG. 1. Rate of ascorbic acid oxidation in 30° C. in pyrex glassware-distilled water, in water from a laboratory still, in tap water, and in glassware-distilled water plus added copper: (1) Glassware-distilled water plus 19.9 mg. of ascorbic acid per l.; (2) water from a laboratory still plus 20.0 mg. of ascorbic acid per l.; (3) tap (hot) water plus 16.5 mg. of ascorbic acid per l.; (4) No. 1 plus 0.15 p.p.m. of copper; and (5) No. 1 plus 0.50 p.p.m. of copper.

Impurities in the water from the hot water tap caused the ascorbic acid to oxidize rapidly. This water contained 0.12 p.p.m. of copper, 0.85 p.p.m. of iron, and, as indicated by the milkiness of the water when it was first drawn, considerable dissolved oxygen. The chlorides test with silver nitrate was positive. The iodine test for chlorine was negative.

The amount of copper in the tap water and in water from the laboratory still was measured by an all-dithizone method using a spectrophotometer (3, 12), and of iron² by reduction with zinc and titration with potassium permanganate (1).

² We are indebted to H. S. Haller of these laboratories for the copper and iron determinations.

Water from the laboratory still contained only a trace of copper (< 0.01 p.p.m.). The chlorides test was negative.

Barron, DeMeio and Klemperer (2) reported that "among the metallic salts tested (Mn, Ni, Fe, Co, Ca, Cu), copper is the only catalyst for the oxidation of ascorbic acid, its catalytic action being noticed in concentrations as small as 46 micrograms of copper per liter." This is equivalent to 0.046 p.p.m. Mapson (11) has shown that the complete removal of copper from a solution renders ascorbic acid stable to O_2 .

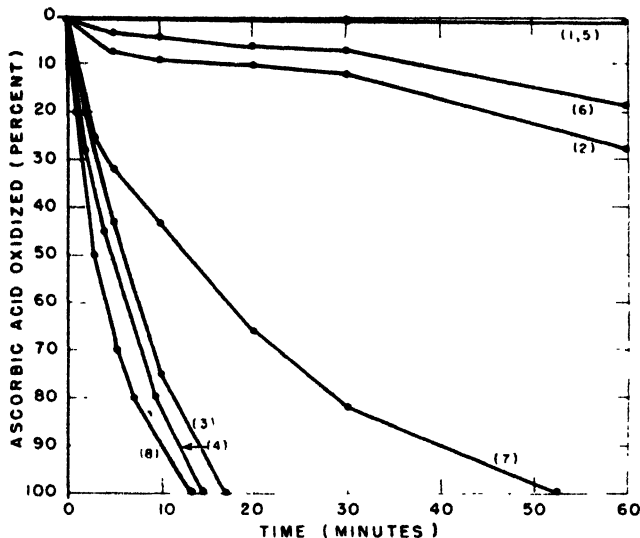


FIG. 2. Effect of deaeration on the rate of ascorbic acid oxidation in pyrex glassware-distilled water at 30° C. when hydrogen peroxide or copper or both were added: (1) Undeaerated glassware-distilled water plus 20.0 mg. of ascorbic acid per l.; (2) same as No. 1 plus 0.03 ml. of 30% H_2O_2 per l.; (3) same as No. 1 plus 0.3 p.p.m. of copper; (4) same as No. 1 plus 0.03 ml. of 30% H_2O_2 per l. and 0.3 p.p.m. of copper; (5) deaerated glassware-distilled water plus 20.0 mg. of ascorbic acid per l. and redeaerated; (6) same as No. 5 plus 0.03 ml. of 30% H_2O_2 per l. after the first deaeration; (7) same as No. 5 plus 0.3 p.p.m. of copper after the first deaeration; (8) same as No. 5 plus 0.03 ml. of 30% H_2O_2 and 0.3 p.p.m. of copper after the first deaeration.

In figure 2 are shown the rates of oxidation of ascorbic acid (20.0 mg./l.) in undeaerated and in deaerated glassware-distilled water, without and with added copper or hydrogen peroxide or both. In preparing the latter solutions, the glassware-distilled water was deaerated by heating it to a temperature (50° C.) at which it would boil when under the vacuum (13); then the vacuum was broken, the warm water poured into a large beaker, a small volume of concentrated ascorbic acid added and the copper or hydrogen peroxide or both, if required, and the solution redeaerated. Thus the fluid was deaerated twice. During the second deaeration and about 30 minutes thereafter the reacting temperature was warmer than 30° C. In order to prevent reincorporation of

air, each 10 ml. for titrating was drawn into a pipette from the deaerating collecting flask.

In the course of an hour there was less than 30 per cent destruction of the ascorbic acid in the undeaerated solution to which hydrogen peroxide had been added. However, the ascorbic acid was oxidized quickly in the presence of as little as 0.3 p.p.m. of copper and at an even faster rate when 0.03 ml. of 30 per cent hydrogen peroxide per liter of water also was added.

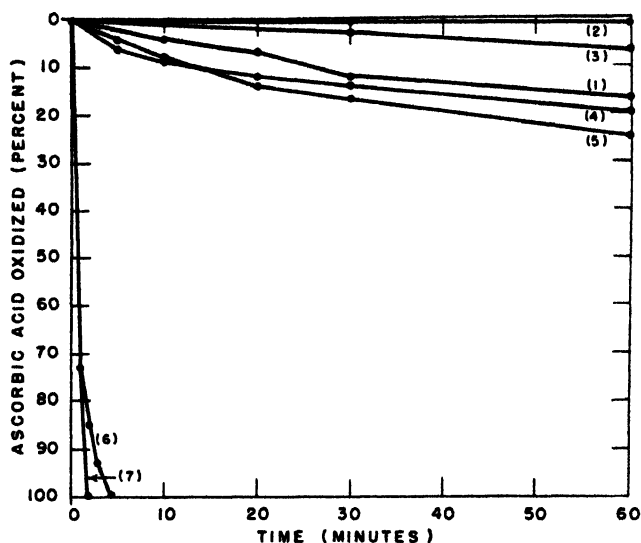


FIG. 3. Rate of ascorbic acid oxidation of 30° C. in fresh raw milk, in pyrex glassware-distilled water, in their mixtures, and in equal parts of milk and water from a laboratory still, and in fresh raw milk with added copper, H_2O_2 , or both: (1) Fresh raw milk containing 16.1 mg. of ascorbic acid per l.; (2) glassware-distilled water plus 20.9 mg. of ascorbic acid per l.; (3) equal parts raw milk and glassware-distilled water plus 12.0 mg. of ascorbic per l.; (4) equal parts of fresh raw milk and water from the laboratory still plus 12.0 mg. of ascorbic acid per l.; (5) No. 1 plus 0.3 p.p.m. of copper; (6) No. 1 plus 0.03 ml. of 30% hydrogen peroxide per l.; (7) No. 1 plus 0.3 p.p.m. of copper and 0.03 ml. of 30% H_2O_2 per l.

In figure 3 are shown curves that illustrate the rate of oxidation at 30° C. of ascorbic acid in fresh raw milk, in glassware-distilled water, and in a 1:1 mixture of the two under comparable temperature, indirect light and air incorporating conditions. Others (6, 15) have shown the rate of destruction of ascorbic acid in milk protected from direct light and from copper contamination.

Also shown in figure 3 are the rates of oxidation of ascorbic acid in a mixture of equal parts of fresh raw milk and of water from the laboratory still, fresh raw milk with 0.3 p.p.m. of added copper, fresh raw milk to which 0.03 ml. of 30 per cent hydrogen peroxide per liter of milk had been added and also fresh raw milk to which both copper and 30 per cent hydrogen peroxide had been added in these proportions.

Early investigators of the oxidized flavor in milk (7, 17) believed that the destruction of ascorbic acid and the development of the oxidized flavor was influenced by an enzyme which occurs naturally in milk. In more recent publications Krukovsky (9) and Krukovsky and Guthrie (10) presented evidence to show that a promotor (an enzyme) of ascorbic acid oxidation in milk by hydrogen peroxide might be responsible for quick conversion of ascorbic acid to dehydroascorbic acid. The presence of an enzyme in the water used in our experiments was precluded by the sources of the water. Copper was a key factor in the oxidation of the acid and, when hydrogen peroxide also was added to the solution, the ascorbic acid oxidized rapidly.

Milk produced by cows on a normal ration contains about 0.15 mg. of copper per liter (5). Milk also contains about 5.4 p.p.m. of oxygen (15).

On the first page of their article, "Quantitative Determination of Dissolved Oxygen", Sharp, Hand and Guthrie (16), in describing the standardization of the dye solution to be used for titrating milk to determine its ascorbic acid content, direct as follows: "Weigh accurately approximately 100 mg. of ascorbic acid, place in a 1-liter volumetric flask, and make up to volume with distilled water. Mix thoroughly and use at once for standardizing." In an earlier publication Sharp (14) directs that the ascorbic acid solution be used at once for standardizing the dye (2,6-dichlorophenolindophenol) solution.

The significance and importance of titrating at once is emphasized by the results presented in figure 1. A dye factor based upon the ascorbic acid content of an unstable solution might be inaccurate. Since ascorbic acid is likely to be used in increasing amounts for retaining the fresh flavor of milk (18), the importance of correctly standardizing the dye solution that is used in measuring the ascorbic acid content of milk becomes apparent.

CONCLUSIONS

Water may contain impurities that accelerate the oxidation of ascorbic acid.

Ascorbic acid is stable for more than an hour in pyrex glassware-distilled water and relatively unstable in glassware-distilled water to which hydrogen peroxide has been added. It is unstable when these solutions contain copper.

Three-tenths p.p.m. of copper is sufficient to cause the rapid oxidation of ascorbic acid in glassware-distilled water.

Only pure water should be used in preparing an ascorbic acid solution that is to be used in standardizing the dye, 2,6-dichlorophenolindophenol, with which the ascorbic acid content of milk and other fluids can be measured in acid solution by direct titration.

REFERENCES

- (1) Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*. 6th Ed. p. 641. 1945.
- (2) BARRON, E. S. G., DeMEIO, R. H., AND KLEMPERER, F. Studies on Biological Oxidations. V. Copper and Hemochromogens as Catalysts for the Oxidation of Ascorbic Acid. The Mechanism of the Oxidation. *J. Biol. Chem.*, 112: 625-640. Jan., 1936.
- (3) BENDIX, G. H., AND GRABENSTETTER, D. A Dithizone Method for the Rapid Determination of Copper. *Ind. Eng. Chem., Anal. Ed.*, 15: 649. 1943.

- (4) BIRCH, T. W., HARRIS, L. J., AND RAY, S. N. A Microchemical Method for Determining the Hexuronic Acid (Vitamin C) Content of Foodstuffs. *Biochem. J.*, **27**: 590-594. 1933.
- (5) ELVEHJEM, C. A., STEENBOCK, H., AND HART, E. B. The Effect of Diet on the Copper Content of Milk. *J. Biol. Chem.*, **83**: 27-34. 1929.
- (6) HENDERSON, J. L. The Vitamin C Content of Milk and Its Relation to Oxidized Flavor. *Assoc. Bull. Intern. Assoc. Milk Dealers*, **12**, 32nd year. Feb., 1940.
- (7) KENDE, S. Untersuchungen über "ölig-talgige," "schmirgelige" Veränderungen der Milch. *Milchwirtschaftliche Forsch.*, **13**: 111-143. 1932.
- (8) KENNY, C. L. A Study of the Thermostability of Vitamin C. Unpublished Ph.D. thesis, Columbia Univ. 1926.
- (9) KRUKOVSKY, V. H. Ascorbic Acid Oxidation in Milk by Preformed Hydrogen Peroxide. *J. Dairy Sci.*, **32**: 163-165. 1949.
- (10) KRUKOVSKY, V. H., AND GUTHRIE, E. S. Vitamin C, Hydrogen Peroxide, Copper and the Tallowy Flavor in Milk. *J. Dairy Sci.*, **29**: 293-306. 1946.
- (11) MAPSON, L. W. Halides and Ascorbic Acid Oxidation. *Biochem. J.*, **35**: 1322. 1941.
- (12) MORRISON, S. L., AND PAIGE, H. L. Modified All-Dithizone Method for Determination of Traces of Copper. *Ind. Eng. Chem., Anal. Ed.*, **18**: 211. 1946.
- (13) MOTTERN, H. A., AND VON LOESECKE, H. W. De-aeration and Flash Pasteurization of Orange and Grapefruit Juices. *Fruit Products J.* **12**, **11**: 325-326. 1933.
- (14) SHARP, P. F. Rapid Method for the Quantitative Determination of Reduced Ascorbic Acid in Milk. *J. Dairy Sci.*, **21**: 85-88. 1938.
- (15) SHARP, P. F., GUTHRIE, E. S., AND HAND, D. B. A New Method for Retarding Oxidized Flavor and Preserving Vitamin C—Deaeration. *Assoc. Bull. Intern. Assoc. Milk Dealers*, No. 20. April, 1941.
- (16) SHARP, P. F., HAND, D. B., AND GUTHRIE, E. S. Quantitative Determination of Dissolved Oxygen. *Ind. Eng. Chem.*, **13**: 593-597. Sept., 1941.
- (17) SHARP, P. F., TROUT, G. M., AND GUTHRIE, E. S. Vitamin C, Copper, and the Oxidized Flavor of Milk. *Tenth Ann. Rept. N. Y. State Assoc. Dairy and Milk Inspectors*, pp. 153-164. 1936.
- (18) WEINSTEIN, B., LOEWENSTEIN, M., AND OLSON, H. C. The Use of Ascorbic Acid in Controlling Oxidized Flavor in Milk. *Milk Plant Monthly*, pp. 116-119. Oct., 1948.

DEFERMENT OF AN OXIDIZED FLAVOR IN FROZEN MILK BY ASCORBIC ACID FORTIFICATION AND BY HYDROGEN PEROXIDE OXIDATION OF THE ASCORBIC ACID OF THE FRESH MILK¹

R. W. BELL AND T. J. MUCHA

Division of Dairy Products Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture, Washington 25, D. C.

Two outstanding reasons why milk has not been preserved in the frozen state on a larger commercial scale are the development during storage of the flavor defect usually known as oxidized and the deterioration in body as evidenced by uneven distribution of insoluble solids in the thawed milk. The former will be considered here.

Krukovsky and Guthrie (3) found that the reaction which produces the "tallowy" (oxidized) flavor could be inhibited by quick and complete photochemical or chemical oxidation of ascorbic acid in the milk to dehydroascorbic acid prior to pasteurization and storage. Their samples were stored at 0 to 5° C. and were examined usually over a period of about a week.

Whether the characteristic oxidized flavor which develops in frozen milk has its origin in the same source as does the oxidized flavor in unfrozen milk is not known. It has been detected in thawed milk after a storage period of only 2 or 3 weeks (1), and the source is thought to be the same.

The questions which the present investigations undertook to answer were how long the onset of the characteristic oxidized flavor could be delayed in frozen storage by chemical (hydrogen peroxide) oxidation of ascorbic acid in the fresh milk, and whether fortification of the fresh milk with ascorbic acid would be as effective.

The general methods that were used have been described previously (1). The milk was pasteurized in stainless steel containers by holding it at 71° C. for not less than 15 seconds, homogenized at 2,500 lb. pressure per square inch, and cooled at once. During this processing the milk was exposed briefly to copper because some of the tin had worn off the short lengths of pipe leading to and away from the homogenizer and off the small surface cooler. The ascorbic acid was added just prior to packaging the milk, the hydrogen peroxide just before or after pasteurization. Fresh samples were held in pyrex flasks at 2 to 4° C.; others were canned and stored in a room that was maintained at -17° C., and examined as indicated in the tables.

The initial ascorbic acid titrations referred to in table 1 were completed within an hour after the samples were prepared, the oxidation-reduction (E_h) measurements within the next 2 hours. The E_h of the fresh milk was low and the milk was well poised. During the next few days in cold storage the ascorbic acid in the samples held at 2 to 4° C. oxidized, the E_h became more positive and

Received for publication May 23, 1949.

¹ This work was done with funds from the Agricultural Research and Marketing Act of 1946.

TABLE 1

Relationship between the amounts of 30 per cent hydrogen peroxide and/or ascorbic acid added to milk after pasteurization and the development of the oxidized flavor in milk subsequently held at 2° to 4° C. and at -17° C.

Holding temperature and time held	Pasteurized control				Ascorbic acid added to (A)				0.03 ml. of 30% H ₂ O ₂ /l. added to (A)				Ascorbic acid added to (D)			
	(A)		(B)		(C)		(D)		(E)		(F)		(G)		(H)	
	A.A. ^a (mg./l.)	E _h ^a (volts)	A.A. (mg./l.)	E _h (volts)	F (mg./l.)	A.A. (mg./l.)	E _h (volts)	F (volts)	A.A. (mg./l.)	E _h (volts)	F (volts)	A.A. (mg./l.)	E _h (volts)	F (volts)	A.A. (mg./l.)	E _h (volts)
2° to 4° C.:																
1 to 3 hr.	13.8	0.206	33.6	0.189		54.0	0.182	1.2	0.246	14.4	0.235	31.2	0.216			
2 d.	0.0	0.259	1.8	0.239		16.8	0.219	0.0	0.257	0.0	0.240	0.0	0.249	ox		
6 d.	0.0	0.316	sl -	0.309	sl		0.308	sl	0.0	0.304		0.0	0.301	ox		
-17° C.:																
14 d.	11.4	0.220	tr	0.198		51.6	0.187	0.0	0.255	0.0	0.243	ox	0.221	ox +		
56 d.	5.4	0.234	st	0.208	ox	26.4	0.190	tr	0.0	0.269	sl +	0.0	0.256	ox	10.2	0.229
Equal parts of (A) and (D)																
Holding temperature and time held	(G)		(H)		(I)		(J)		(K)		(L)		(M)		(N)	
	(G)		(H)		(I)		(J)		(K)		(L)		(M)		(N)	
	A.A. ^a (mg./l.)	E _h ^a (volts)	A.A. (mg./l.)	E _h (volts)	F (mg./l.)	A.A. (mg./l.)	E _h (volts)	F (volts)	A.A. (mg./l.)	E _h (volts)	F (volts)	A.A. (mg./l.)	E _h (volts)	F (volts)	A.A. (mg./l.)	E _h (volts)
2° to 4° C.:																
1 to 3 hr.	4.8	0.230	23.4	0.209		41.4	0.195	3.0	0.240	6.6	0.232					
2 d.	0.0	0.259	0.0	0.245	sl	1.8	0.238	sl	0.0	0.270		0.0	0.243			
6 d.	0.0	0.305	tr	0.301	sl	0.0	0.299	sl +	0.0	0.304	sl	0.0	0.310	sl		
-17° C.:																
14 d.	0.0	0.229	sl	0.217	ox	26.8	0.204	ox	0.0	0.242	ox	0.0	0.244	ox		
56 d.	0.0	0.249	ox +	0.255	ox	22.8	0.209	ox	0.0	0.254	ox -	0.0	0.251	ox		

^a A.A. = ascorbic acid; E_h = oxidation-reduction potential at 30° C.; F = flavor. Oxidized flavor intensity is indicated as follows: tr = trace, sl = slight, ox = oxidized, st = strongly oxidized. Where there are no flavor notations the samples were considered satisfactory.

some of the samples developed a trace to an almost strong oxidized flavor. As was anticipated by the findings of Krukovsky and Guthrie (3) the milk *D* to which 0.03 ml. of 30 per cent hydrogen peroxide per liter of milk was added did not become oxidized in flavor; samples of this same milk to which ascorbic acid was added (*E* and *F*) did, *F* to a greater intensity than *E*, even though it had a lower initial E_h .

After 14 days at -17° C. the thawed control sample was just beginning to taste oxidized; *B* and *C*, ascorbic acid fortified milks, had a normal flavor; *D*, to which hydrogen peroxide was added, was unchanged; *E* and *F* tasted oxidized. Of the remaining five milks, only *J* was judged normal. However, after 56 days at -17° C. all of the milks in the series including *C* and *D* were oxidized to a greater or lesser degree; *C*, which originally contained 54 mg. of ascorbic acid per liter of milk, being less oxidized than *D*.

It is well known that copper catalyzes changes in milk which cause the oxidized flavor. The data in table 2 re-emphasize this fact and are evidence that hydrogen peroxide delays, but does not prevent, the development of this off flavor in frozen milk. Hand drawn, uncooled morning milk was utilized. The milk was divided into three parts. One (*A*) was pasteurized, homogenized and cooled as usual. The ascorbic acid content of the fresh, raw milk was 21.2 mg./l., that of the pasteurized product immediately after being cooled was 20.6 mg./l. In preparing the second part (*B*) 0.03 ml. of 30 per cent hydrogen peroxide was added per liter of raw milk. In a few minutes the milk contained only 2.1 mg. of ascorbic acid per liter; after pasteurization, it contained none. Part three (*C*) was prepared by first combining equal parts of fresh raw milk and fresh raw milk to which 0.03 ml. of 30 per cent hydrogen peroxide per liter had just been added. Finally, copper in the form of copper sulphate was added at the rate of 1.0 p.p.m. to each of these three pasteurized milks, thus forming milks *D*, *E* and *F*. The ascorbic acid content and E_h of these six milks were determined within 3 hours after the samples were placed in 2 to 4° C. storage.

The "heated" flavor noted in table 2 ought not to be interpreted as "cooked." It was of mild intensity and not readily detectable. At one stage it was described as slight almond. In that part of this experiment (*B*) in which 30 per cent hydrogen peroxide was added at the rate of 0.03 ml./l. of raw milk, the onset of the oxidized flavor was deferred longer than 55 days. The oxidized flavor that developed in milks *D*, *E* and *F* was particularly objectionable. It was unclean and somewhat rancid.

Table 3 shows the relationship between the amounts of ascorbic acid added to pasteurized milk and of 30 per cent hydrogen peroxide added to the milk before or after pasteurization and the development of the oxidized flavor under two conditions of storage. Heavy fortification with ascorbic acid protected the flavor of the milk more effectively than did the addition of 30 per cent hydrogen peroxide.

In experiment 4, as reported in table 4, pasteurized milk was fortified heavily with ascorbic acid, and 30 per cent hydrogen peroxide was added to separate

TABLE 2
Relationship between the addition of 30 per cent hydrogen peroxide to milk before pasteurization, copper after pasteurization, and the development of the oxidized flavor in the pasteurized milk subsequently held at 2° to 4° C. and at -17° C.

Holding temperature and time held	Pasteurized control		0.03 ml. of 30% H ₂ O ₂ /l. of milk		Equal parts of (A) and (B)		Copper added 1.0 p.p.m.					
	(A)		(B)		(C)		Added to (A)		Added to (B)		Added to (C)	
	A.A.* (mg./l.)	E _n * (volts)	A.A. (mg./l.)	E _n (volts)	F ^a	A.A. (mg./l.)	E _n (volts)	F (mg./l.)	A.A. (mg./l.)	E _n (volts)	F (mg./l.)	F (volts)
2° to 4° C.:												
2 to 3 hr . . .	18.0	0.239	0.0	0.264	heated	7.8	0.257	15.7	0.279	0.0	0.298	0.0
2 d.	0.0	0.306	0.0	0.312	{ sl, heated	0.0	0.306	0.0	0.316	0.0	0.308	0.0
5 d.					} almond							heated
-17° C.:												
33 d.	0.0	0.283	sl	0.272		0.0	0.264	sl -	0.0	0.299	st	0.293
55 d.		0.306	st	0.280	heated		0.288	ox	0.314	0.318	st	0.306
90 d.					ox			st				ox -

* Flavor and other designations as in table 1.

TABLE 3

Relationship between the amounts of ascorbic acid added to pasteurized milk and of 30 per cent hydrogen peroxide added to milk before or after pasteurization and the development of the oxidized flavor in milk subsequently held at 2° to 4° C. and at -17° C.

Holding temperature and time held	Pasteurized control		Ascorbic acid added to (A)		0.03 ml. of 30% H ₂ O ₂ /l. of raw milk		Equal parts of (A) & (D) after pasteurization		0.06 ml. of 30% H ₂ O ₂ /l. of raw milk		Equal parts of (A) & (F) after pasteurization		0.03 ml. of 30% H ₂ O ₂ /l. of pasteurized milk		Equal parts of (A) and (H)		0.06 ml. of 30 per cent H ₂ O ₂ /l. of pasteurized milk		Equal parts of (A) and (J)	
	(A)	(B)	(C)	(D)	(E)	(F)	(G)	(H)	(I)	(J)	(K)	(L)	(M)	(N)	(O)	(P)	(Q)	(R)	(S)	(T)
	A.A. ^a (mg./l.)	F ^a (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)	A.A. (mg./l.)	F (mg./l.)
2° to 4° C.:																				
2 to 4 hr.	14.4	65.4	117.6	0.0	6.6	0.0	1.2	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5 d.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-17° C.:																				
27 d. .	0.0	st 37.8	tr 75.0	0.0	sl 0.0	ox 0.0	ox 0.0	st 0.0	ox - 0.0	sl 0.0	ox - 0.0	st 0.0	ox - 0.0	ox - 0.0	sl 0.0	ox - 0.0	st 0.0	ox - 0.0	st 0.0	ox - 0.0
47 d.		st 34.1	ox 70.0	sl 0.0	ox 0.0	ox 0.0	ox 0.0	st 0.0	ox - 0.0	st 0.0	ox - 0.0	st 0.0	ox - 0.0	ox - 0.0	st 0.0	ox - 0.0	st 0.0	ox - 0.0	st 0.0	ox - 0.0

^a Flavor and other designations as in table 1.

portions of the raw milk in carefully graduated amounts rather than by dilution in sequence of milk containing a large amount of added hydrogen peroxide with normal milk. Immediately after each addition of hydrogen peroxide the amount of ascorbic acid was 15.5, 3.7, 4.9, 7.0 and 16.5 mg./l., and of dehydroascorbic acid it was 8.2, 19.8, 18.1, 16.2 and 7.3 mg./l., respectively. Thus the total vitamin C at this time, regardless of the amount of hydrogen peroxide that was added, was approximately the same.

The initial data in the table were obtained 2 to 4 hours after the milks had been pasteurized and stored. Five days later, among the samples maintained at 2 to 4° C., only *A*, *B*, *C* and *D* contained any vitamin C. It was the oxidized form (2). Sample *C*, the most heavily ascorbic acid-fortified milk, had a normal flavor at this time. Samples *D* to *H*, inclusive, tasted old and somewhat stale, but not oxidized.

After 27 days at -17° C., only thawed samples *B* and *C* contained ascorbic acid. Samples *A*, *B* and *C* contained 3.5, 4.9 and 7.0 mg. of dehydroascorbic acid per liter, respectively; the other samples contained none. Sample *A* tasted oxidized, *B* slightly oxidized, and *C*, the most heavily ascorbic acid fortified milk, had a normal flavor. Samples *D* to *H*, inclusive, to which 30 per cent hydrogen peroxide had been added at the rate of 0.015 to 0.150 ml./l. of raw milk, were oxidized or strongly oxidized. *G* and *H* also had a slight but definite almond flavor due to the relatively large amounts of hydrogen peroxide that were added to the raw milk.

Twelve days later the samples were found to have changed in the usual way. The vitamin C content of *A*, *B* and *C* was decreasing, their E_n was increasing and, except for *C*, the intensity of the oxidized flavor was the same or somewhat greater. *C* no longer had a normal flavor; an oxidized flavor was detectable.

In this experiment, as well as in others in which 30 per cent hydrogen peroxide was added and a strong oxidized flavor developed, the flavor was also tallowy. These samples were more objectionable than were those which, although fortified with ascorbic acid, became oxidized or strongly so.

DISCUSSION AND CONCLUSIONS

It ought not to be implied from the work of Krukovsky and Guthrie (3, 4) on market milk stored at 0° to 5° C. that a rapid, complete oxidation of vitamin C in milk by hydrogen peroxide prevents the development of an oxidized flavor indefinitely. The results reported here indicate that, when such milk is preserved in frozen storage, this characteristic off flavor will be detectable eventually. From a chemical standpoint this is to be expected, if the flavor is due to a mild oxidative reaction. Whenever a physico-chemical system tends to have a higher oxidation-reduction potential, the more easily oxidizable constituents (for example, ascorbic acid in milk) will tend to oxidize. Free oxygen is not essential because oxidative changes can take place without its participation, merely by transfer of electrons from reductants to oxidants. So long as this can go on, as it can even in frozen milk, the system will be unstable and tend toward a higher poten-

tial. The addition of ascorbic acid to milk lowers the oxidation-reduction potential, the milk is more static and its tendency to develop the oxidized flavor is decreased.

Ascorbic acid, a strong reducing agent, is almost ideal for lowering the E_h of milk. It is a natural constituent of milk; it is one of two equally biologically active forms of vitamin C; it does not alter the flavor, nor does it appreciably increase the acid intensity of the system. Furthermore, it is inexpensive. However, its tendency to oxidize is relatively great. Added to market milk, ascorbic acid is effective in delaying, and usually in preventing, the onset of the oxidized flavor during the life of the product. In frozen milk, which has a longer commercial life than market milk, ascorbic acid defers or delays but does not prevent the defect. This is also true of hydrogen peroxide when it is added to milk that is to be preserved in a frozen state.

REFERENCES

- (1) BELL, R. W. Retention of Ascorbic Acid, Changes in Oxidation-Reduction Potential, and the Prevention of an Oxidized Flavor during Freezing Preservation of Milk. *J. Dairy Sci.*, **31**: 951-960. 1948.
- (2) GUNSALUS, I. C., AND HAND, D. B. The Use of Bacteria in the Chemical Determination of Total Vitamin C. *J. Biol. Chem.*, **141**: 853-858. 1941.
- (3) KRUKOVSKY, V. N., AND GUTHRIE, E. S. Ascorbic Acid Oxidation a Key Factor in the Inhibition or Promotion of the Tallowy Flavor in Milk. *J. Dairy Sci.*, **28**: 565-579. 1945.
- (4) KRUKOVSKY, V. N., AND GUTHRIE, E. S. Vitamin C, Hydrogen Peroxide, Copper and the Tallowy Flavor in Milk. *J. Dairy Sci.*, **29**: 293-306. 1946.

THE RELATION OF PEDIGREE PROMISE TO PERFORMANCE OF PROVED HOLSTEIN-FRIESIAN BULLS¹

FRANKLIN E. ELDRIDGE² AND G. W. SALISBURY³

*Laboratory of Animal Breeding and Artificial Insemination,
Department of Animal Husbandry, Cornell University, Ithaca, New York*

The items of information which are used as criteria for selection of dairy bulls normally become available in the following time sequence: (a.) pedigree, (b.) individuality, (c.) progeny performance.

The progeny test is theoretically the most accurate method for estimating the production of future daughters of a bull and consequently has been accepted generally as the most accurate from a practical standpoint also. Recent studies by Beardsley (1) indicate that the predictability of the productive performance of a group of daughters in a second herd from the performance of a group of daughters in a first herd is not especially high, the correlation coefficient being 0.34. Thus, except for situations where a proved sire is to be bred in the future to cows known to have the same general genetic make-up and to be kept under identical conditions to those cows with which he was first proved, the accuracy of prediction from first proof is not as high as generally implied.

Even though, for a particular situation, reliance were to be placed solely on the proved sire, all young bulls must be selected on some other basis. Individuality of a bull should be used as a basis of selection to improve milk production or butterfat yield only to the extent that type in the male is correlated with milk production in his daughters. So far as is known, no adequate test of such an hypothesis has been made. Thus, in selecting young dairy bulls, reliance must be placed largely on pedigree information. It has been difficult to determine the effectiveness of pedigree selection in improving the productive performance of dairy cattle, since few data on this question have been reported.

Gowen (5, 6) and Copeland (2, 3), using Advanced Registry or Register of Merit records, have determined the correlations that existed between the average production of the daughters of bulls and different groups of their relatives considered separately. Madsen (9) determined similar correlations from data more comparable to that obtained in D.H.I.A. or H.I.R. testing programs, but he used different groups of bulls and relatives to obtain each correlation.

Lush and Schultz (8) studied the relation between pedigree promise as indicated by Advanced Registry records and the performance of daughters of

Received for publication May 25, 1949

¹ The data in this paper are from a thesis submitted by the senior author to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Feb., 1948. The authors are indebted to Professor S. J. Brownell, Project Leader, Animal Husbandry Extension, New York State College of Agriculture, for permission to obtain these data from the Herd Analysis Extension Project.

² Now at Kansas State College, Manhattan.

³ Now at University of Illinois, Urbana.

bulls when tested in D.H.I. Associations. Here again different groups of individuals were used to study the relationship of the production of a certain ancestor to the final proof of the sires. These authors point out that the most satisfactory evidence should be obtained from multiple correlation studies where data were available on all groups of ancestors for the bulls under consideration.

The purpose of the study published here was to develop a method and estimate its accuracy for selecting an unproved bull, using the production performance of the ancestors, collateral females and mates, when the information was complete for each pedigree.

EXPERIMENTAL

There were 1,451 Holstein-Friesian bulls proved by D.H.I.A. records in New York prior to Feb. 1, 1947. A number of these proved bulls were sons of proved sires and were born in herds which were cooperating in the New York Dairy Extension Herd Analysis Project. As a consequence, records of butterfat production for relatives on both the paternal and maternal side of the pedigree were available for study.

Two series of proved bulls were selected from those born in analyzed herds. Series A included 158 bulls (average number daughter-dam comparisons, 8.40), each of which had the following recorded information available, in addition to the proof on his sire (average number of daughter-dam comparisons, 21.05)⁴: a. The dam of the bull had at least one record (average number, 4.64), and b. The dam of the bull had at least one daughter with a record (average number of daughters, 1.85).

Series B included 141 bulls (average number daughter-dam comparisons, 8.13), each of which had the following recorded information available in addition to the proof on his sire (average number daughter-dam comparisons, 21.81)⁵: a. The dam of the bull had at least one record (average number, 4.33), and b. The dam of the bull was by a proved sire (average number daughter-dam comparisons, 18.07).⁶

Of the total of 207 bulls meeting either set of established criteria for study, 92 satisfied both sets of requirements and were included in both series. Thus, the two series are not independent samples and any comparisons between the results of the two series should not be made without recognizing the correlation that exists between them.

The average of all butterfat records for each cow, calculated to a mature equivalent, 305-day lactation, twice-a-day milking basis, by D.H.I.A. standards, was used as a measure of the producing ability of each cow. The variables for each bull were means of the production of groups of females in each case, except for the production of the bull's dam which was an average, in most cases, of several

⁴ Included among the sires of bulls in series A were 6 bulls with 4, and 7 bulls with 3 daughter-dam comparisons. All others had 5 or more.

⁵ Included among the sires of bulls in series B were 1 bull with 4, and 4 bulls with 3 daughter-dam comparisons.

⁶ Included were 6 sires having 4, and 17 sires having 3 daughter-dam comparisons, the dam of the series B bulls being one of these daughters in each case.

records of the same individual. The figure for a particular variable of all bulls was given equal weight regardless of the number of production records from which the figure was calculated.

The variables for this study were assigned symbols as follows: X_1 = average production of the mates of the proved bull; X_2 = average production of the maternal half-sisters of the proved bull; X_3 = average production of the dam of the proved bull; X_4 = average production of the paternal half-sisters of the proved bull; X_5 = average production of the dams of the paternal half-sisters of the proved bull; X_6 = average production of the daughters of dam's sire (paternal half-sisters of the bull's dam), and Y = average production of the daughters of the proved bull.

Simple correlation coefficients were calculated between all pairs of the above variables (table 1) to determine multiple regression equations after the methods outlined by Snedecor (11), in order to establish the optimum weight to be given each variable in predicting the level of production of Y , the daughters of the proved bulls.

RESULTS

The mean production of the relatives of the bulls and the correlations between these relatives are shown in table 1. The average production of the bulls' dams was from 30 to 80 lb. higher than the average production of the animals constituting any other variable, indicating that the dams were a selected group, not a random group of females from this population. This must be borne in mind in interpreting the results of this investigation.

TABLE 1

Mean production in pounds of butterfat for each group of relatives in both Series A and Series B and the correlations between these groups within each series

	Series	Mates (X_1)	Maternal half sisters (X_2)	Dam (X_3)	Paternal half sisters (X_4)	Dams of paternal half sisters (X_5)	Daughters of maternal grand sire (X_6)	Daughters of bull (Y)
Mean pro- duction and standard deviation	A	380 ± 54	421 ± 74	460 ± 78	408 ± 54	402 ± 53		387 ± 65
	B	382 ± 50		455 ± 78	422 ± 50	412 ± 52	406 ± 53	394 ± 63
X_1	A		0.18*	0.18*	0.26**	0.23**		0.66**
	B			0.17*	0.18*	0.08	0.15*	0.65**
X_2	A			0.32	0.47**	0.42**		0.22**
	B							
X_3	A				0.34**	0.47**		0.15
	B				0.25**	0.29**	0.48	0.21*
X_4	A					0.67**		0.35**
	B					0.64**	0.40**	0.26**
X_5	A							0.18*
	B						0.63**	0.11
X_6	A							
	B							0.26**

* Indicates significance at the 5 per cent level of probability.

** Indicates significance at the 1 per cent level of probability.

The complete regression equation for predicting the average production of the daughters of the bulls. Using the correlations of table 1 as the basis for calculation, the following regression equations were developed.

$$(1) \quad \hat{Y}_A = 30 + 0.75^{**}X_1 + 0.03X_2 + 0.01X_3 + 0.34^{**}X_4 - 0.21^*X_5$$

$$R = 0.701^{**} \qquad R^2 = 0.491$$

$$(2) \quad \hat{Y}_B = 0 + 0.75^{**}X_1 + 0.01X_3 + 0.23^*X_4 - 0.22X_5 + 0.24^*X_6$$

$$R = 0.689^{**} \qquad R^2 = 0.475$$

(**Significant at the 1 per cent level of probability; *significant at the 5 per cent level of probability).

The multiple correlation coefficient was highly significant in each equation. The square of these coefficients indicated that from about 47 to 49 per cent of the total variance among the averages of the daughters of different bulls was associated with the regression plane described by these equations.

The effect of the production level of the mates of the bulls. The correlation between Y , the average production of the daughters of a bull, and X_1 , the average production of their dams or the mates of the bull, was +0.66 for Series A and +0.65 for Series B (table 1). Thus, about 43 to 44 per cent of the total variance among the bulls, in the average butterfat production of their daughters, was associated with the variance among the production averages of their respective groups of mates. The other four variables in each series accounted for only about 5 per cent more of the variance among the bulls in the average production of their daughters than was accounted for by the mates alone. However, when tested statistically, this increase of about 5 per cent was found to be highly significant for Series A and significant for Series B.

The effect of the production level of the dams of the bulls. The record on the dam (X_3) could have been removed from the prediction equation in either series without substantially affecting any of the foregoing results. However, this would have implied that the record of the dam had no significance in the selection of a bull. As was pointed out previously, the bulls in this study were from a selected group of dams and, therefore, the population of bulls to which this equation could be applied most justifiably would be one in which the dams were already a selected group of cattle. There are no data within this study with which to evaluate properly the record of the dam in selecting a bull.

The effect of the production level of the maternal half-sisters of the bulls. In Series A the records on the bulls' maternal half-sisters, X_2 , were deleted without affecting the prediction value of the equation as indicated below.

$$(3) \quad \hat{Y}_{A-X_2} = 32.3 + 0.75X_1 + 0.01X_3 + 0.36X_4 - 0.21X_5$$

$$R = 0.700^{**} \qquad R^2 = 0.490$$

The lack of significance of the maternal half-sisters' records was assumed to be due to the fact that there was an average of only 1.85 *maternal* half-sisters per bull. This is in contrast to the average of over 20 *paternal* half-sisters per bull. The theoretically perfect correlation between the average production of eight daughters of a bull and the average production of two of his maternal half-sisters is only +0.27. Thus, it is not surprising, when the environmental variation is

considered, to find that the average production of so few maternal half-sisters had no significant value in discriminating between bulls.

The effect of the production performance in the pedigrees of the bulls. In order to obtain a measure of the accuracy with which the average butterfat production of the daughters of unproved bulls could be estimated from information on the production of the bulls' female relatives before the production of his mates was known, multiple regression equations were developed with X_1 (the production level of the mates) deleted.

$$(4) \quad \hat{Y}_{A-X_1} = 212 + 0.01X_2 + 0.05X_3 + 0.47^{**}X_4 - 0.17X_5 \\ R = 0.369^* \quad R^2 = 0.136$$

$$(5) \quad \hat{Y}_{B-X_1} = 212 + 0.06X_3 + 0.39^{**}X_4 - 0.35^*X_5 + 0.33^*X_6 \\ R = 0.370^* \quad R^2 = 0.137$$

Approximately 14 per cent of the variance among the bulls, in the butterfat production averages of their daughters, was accounted for by means of equations 4 and 5. This reduction of variance was not large but was found to be significant at the 5 per cent level of probability. Such a small correlation coefficient may appear discouraging to breeders, but it must be recognized that there was a large amount of unaccountable, environmental variation within these data.

DISCUSSION

A significant portion of the differences in average production between the bulls' daughter-groups was determined by consideration of the production information available on the females in the bulls' pedigrees, either alone, as in equations 4 and 5, or in combination with the average production of the herd in which he is to be used, equations 1 and 2.

Production information limited to that found in the bulls' pedigrees has been shown to account for 14 per cent of the variance among bulls in the production averages of their daughters. This same information, when added to the information on the production of the mates, accounted for only a 5 per cent increase over the variance accounted for by the mates alone. This discrepancy of approximately 9 per cent was due to the positive correlation which existed between the production average of the mates and the other four independent variables. The multiple regression of X_1 on the four other variables was determined and the multiple correlation coefficients and their squares were found to be as follows:

Series A	$R = 0.288^*$	$R^2 = 0.083$
Series B	$R = 0.251$	$R^2 = 0.063$

Though only approaching the 5 per cent level of significance, these correlation coefficients are indicative of assortative mating and similarities of environment between herds producing and herds using these sires. Because such conditions are not controllable in accumulated data, the correlation coefficients between the several variables in this study may be biased in unknown degree. This condition represents the situation in practice and emphasizes the need for controlled experiments in this field.

On the other hand, it is desirable to derive as much information as possible from the presently available data. Thus, the standard partial regression coefficients (table 2) can be compared with respect to their relative sizes and conse-

TABLE 2
Standard partial regression coefficients for the two series

Series A	Series B
$b'Y1 \cdot 2345 = +0.62$	$b'Y1 \cdot 3456 = +0.60$
$b'Y2 \cdot 1345 = +0.04$	
$b'Y3 \cdot 1245 = +0.01$	$b'Y3 \cdot 1456 = +0.01$
$b'Y4 \cdot 1235 = +0.28$	$b'Y4 \cdot 1356 = +0.19$
$b'Y5 \cdot 1234 = -0.17$	$b'Y5 \cdot 1346 = -0.18$
	$b'Y6 \cdot 1345 = +0.20$

quent effect on the estimated average production of the daughters of a bull (11). The production average of the paternal half-sisters of the bull has the greatest influence, followed rather closely by the production averages of the dams of these half-sisters and the production average of the paternal half-sisters of the bull's dam. Of minor effect are the production averages of the dam and of the maternal half-sisters. From a practical point of view these findings emphasize the proved sire program, since the important items as just cited are in each case parts of sire proofs. From a theoretical point of view the relative importance of the regression coefficients is probably a reflection of differences in numbers comprising the average in each case and differences in selection pressure within each of these groups. The insignificant size of the partial regression coefficient on the dam's record can be explained in one of two ways: (a) the selection of cows for dams of future herd sires is on false premises, or (b) the selection of dams is so effective that relatively little genetic variance exists among them. The true answer is probably a combination of these two explanations.

In the application of these findings to practical sire selection it is evident that the best combination of criteria seems to be a son of a desirably proved sire out of a high producing dam from a desirably proved sire. This combination is already being used by many breeders, but a large proportion of sires are still being selected with the main emphasis on the dam's record. Further study from unselected cow populations will be necessary to determine the true, relative value to be placed upon the record of the dam.

Selection of bulls on the basis of information on production records of direct and collateral female relatives in the immediate pedigree will result in a greater number of successes than random selection, as indicated by the multiple correlation coefficients for equations 4 and 5. Using this correlation, approximately +0.35, and Pearson's "Table for finding the volume of the normal bivariate surface" (10) the following estimates were made:

- (a.) If predictions were made on 100 young bulls using equations 4 or 5, and the highest 50 bulls were selected from 100 prospects, 31 of them, or 62 per cent could be expected on the average to produce daughters whose mean production would exceed the mean of the population.

- (b.) If 21 bulls were selected from 100 prospects on the same basis, 15 of them, or 70 per cent could be expected to have daughters which would exceed the mean production of the population.

Thus, if all young bulls were evaluated on the basis of the equations formulated in this study in addition to the attention that has always been paid to the dam, and then, for example, only the upper 20 per cent selected for herd sires, a general increase in the average production of dairy cattle could be expected.

This study indicates that under existing circumstances of D.H.I.A. testing the predictability of future average performance of bulls' daughters can be made with approximately the same precision from adequate information on the production of direct and collateral female relatives in the pedigree as can be made on the basis of proof in a first herd (1). This indication is not in agreement with the expected theoretical results assuming that production performance is completely hereditary and influenced by additive genes only. In such a case the average of three or more daughters of a bull would be equivalent or superior to the most perfect set of information possible from the pedigree (7). Since, however, the two studies were based on actual results, both complicated by unmeasurable environmental variations, the comparisons are more applicable to the practical situation facing the average dairyman than would be the theoretical comparisons. When the data become available on a series of bulls, each of which has two or more daughters tested in each of a number of herds, it will then be possible to more accurately account for the portion of the variance directly attributable to environment and consequently obtain a more refined measure of the genetic variance.

SUMMARY AND CONCLUSIONS

The average butterfat production of the daughters of 207 proved Holstein-Friesian bulls was studied in relation to the average butterfat production of the mates of these bulls and that of their direct and collateral female relatives. Two series of multiple regression equations were calculated depending on the records of production available: A for 158 bulls, B for 141 bulls, of which 92 bulls were common to each group. A multiple correlation coefficient of approximately + 0.37 was found in both cases between the direct pedigree estimate and average production of the daughters of the bulls, accounting for approximately 14 per cent of the variance among the daughter groups in their butterfat production. When average butterfat yield of the bulls' mates (the dams of the daughters) was added to the information from the pedigrees the correlation coefficients were approximately + 0.7 and accounted for from 47.5 to 49 per cent of the variance between the butterfat averages of the daughters of the bulls. Non-genetic correlations, presumably due to environmental similarities between herds, contributed to the relationships found.

The study indicates that for selection of young dairy bulls, records of performance of females in their pedigrees are of importance in the following order: (a) the average production of the paternal half-sisters of the bull, (b) the average production of the dams of the paternal half-sisters of the bull, and (c)

the average production of the paternal half-sisters of the bull's dam. The average production of the bull's own dam or of his maternal half-sisters showed no relationship to the average production of his daughters. However, since the dams of the bulls were a highly selected group, the only general conclusion that could be made was that among bulls whose dams average above 450 lb. of butterfat, 2X, M.E., D.H.I.A. conditions, the differences between the records of such dams are of little significance.

REFERENCES

- (1) BEARDSLEY, J. P. The Repeatability of Proved Sires and the Curvilinearity of Heritability of Butterfat Production. Thesis, Cornell Univ. 1948.
- (2) COPELAND, L. The Contribution of the Dam in Inheritance of Milk and Butterfat. *J. Dairy Sci.*, 14: 379-393. 1931.
- (3) COPELAND, L. Pedigree Analysis as a Basis of Selecting Bull Calves. *J. Dairy Sci.*, 17: 93-102. 1934.
- (4) DICKEY, H. C., AND LABARTHE, P. Predicting the Transmitting Ability of Young Dairy Sires for Milk Production, Butterfat Test, and Butterfat Production. *J. Dairy Sci.*, 28: 893-900. 1945.
- (5) GOWEN, J. W. Studies in Milk Secretion. XVI. Progeny Performance of Guernsey Sires' Sons. *Ann. Rep., Maine Agr. Expt. Sta., Bull.* 327: 197-252. 1925.
- (6) GOWEN, J. W. The Influence of Inheritance and Environment on the Milk Production and Butterfat Percentage in Jersey Cattle. *J. Agr. Research*, 49: 433-465. 1934.
- (7) LUSH, J. L. *Animal Breeding Plans*. 3rd ed. The Iowa State College Press, Inc., Ames. 1945.
- (8) LUSH, J. L., AND SCHULTZ, E. N. Pedigree Promise and Progeny Test Among Sires Proved in Iowa Cow Testing Associations. *J. Dairy Sci.*, 21: 421-432. 1938.
- (9) MADSEN, K. Inheritance of Milking Capacity. *Nature*, 129: 165-166. 1932.
- (10) PEARSON, K. *Tables for Statisticians and Biometricians, Part II*. Table VII. Table for Finding the Volume of the Normal Bivariate Surface. Positive Correlation. Biometrie Lab., Univ. Coll., London, Cambridge Univ. Press, pp. 78-109. 1931.
- (11) SNEDECOR, G. W. *Statistical Methods Applied to Experiments in Agriculture and Biology*. 4th ed. The Iowa State College Press, Inc., Ames. 1946.

BACTERIOLOGICAL STUDIES OF BOVINE SEMEN. II. THE INCIDENCE OF SPECIFIC TYPES OF BACTERIA AND THE RELATION TO FERTILITY^{1, 2}

P. W. PRINCE, J. O. ALMQUIST AND J. J. REID

*Departments of Dairy Husbandry and Bacteriology
The Pennsylvania State College, State College*

Various types of bacteria have been observed in bull semen collected with the artificial vagina. Hatziolos (10) reported that cocci, spore-forming rods and organisms of the coliform, proteus and pseudomonas groups were the main types of organisms present. Gunsalus *et al.* (9) stated that diphtheroids, staphylococci, *Pseudomonas pyocyaneus* and *Escherichia coli* were the predominant types of bacteria in freshly collected bull semen. A report by the United States Department of Agriculture (14) indicated that diphtheroids were the predominating type of organisms. Staphylococci were found to occur next in frequency and *Pseudomonas aeruginosa* and coliform organisms were present occasionally. Ognianov (12) found *Bacillus subtilis*, *Staphylococcus* and *E. coli*. More recently Edmondson *et al.* (6) isolated streptococci, staphylococci, micrococci, pseudomonas, bacilli, actinomyces and *E. coli* from bovine semen.

There is some evidence that certain types of bacteria can produce toxins and metabolic end products which may be harmful to spermatozoan livability. Several workers (11, 15, 19) have reported observations and opinions supporting this hypothesis but only limited data from controlled experiments are available. Uchigaki (16) found that *E. coli* had a marked deleterious effect on the livability of spermatozoa of the albino rat. Ognianov (12) reported that *E. coli* also was injurious to semen by causing agglutination of bull spermatozoa. Edmondson *et al.* (6) found that hemolytic types of bacteria present in semen caused a decrease in spermatozoan motility during storage. These workers also found that the livability of certain samples containing non-hemolytic types was not as great as the control samples, while in others livability was greater than in the controls.

Perhaps more significant to impaired fertility than the effect of bacteria upon the spermatozoa is infection of the female genital tract by certain organisms following insemination with contaminated semen. There is ample evidence (3, 5, 7, 17, 18) that streptococci and members of the coliform group frequently are associated with ovaritis, metritis, sterility and abortion in females. Although *P. aeruginosa* is generally considered as one of the less virulent of the pathogenic types of bacteria, under favorable conditions the organisms might be capable of establishing infection. Gunsalus *et al.* (8) stated that bulls harboring

Received for publication June 7, 1949.

¹ Authorized for publication May 31, 1949 as paper no. 1521 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

² The data contained in this paper are part of a thesis submitted by the senior author to the Graduate School of the Pennsylvania State College in partial fulfillment of the degree of Master of Science, 1948.

P. aeruginosa in their reproductive tracts are apt to have poor breeding efficiencies and be poor risks for use in artificial breeding. Pounden *et al.* (13) investigated a series of abortions by females previously inseminated with semen from the same bull. Organisms resembling *Staphylococcus albus* were found in the semen of the bull and appeared to be identical to those obtained in large numbers from the pus and exudate surrounding the aborted feti.

The present study was designed to determine the incidence of *P. aeruginosa*, coliform organisms and *Streptococcus* sp. in semen from bulls of known fertility. It also was desired to characterize the predominant types of bacteria present in freshly collected semen and to study their possible relationship to fertility.

METHODS

Semen samples for these studies were obtained from Guernsey, Holstein and Jersey bulls located at the Western Pennsylvania Artificial Breeding Cooperative, Clarion. Previously described collection and shipping techniques (1) were employed in handling the semen samples.

The incidence of *P. aeruginosa* was determined by inoculating tubes of a synthetic asparagine-mineral salts broth with 1 ml. portions of 1 : 10, 1 : 100, 1 : 1,000 and 1 : 10,000 dilutions of semen. A confirmatory test for *P. aeruginosa* was conducted on all asparagine broth tubes that showed turbidity and the presence of a greenish-yellow pigment after 14 days of incubation at 20° C. Confirmation was based on the ability of gram-negative rods to lyse bovine erythrocytes, to liquefy gelatine with the development of a soluble green pigment and to grow at 37° C.

The presence and approximate numbers of members of the coliform group were detected by inoculating lactose with suitable serial dilutions of semen. All positive and doubtful reactions were subjected to standard confirmatory tests (2).

Blood agar plates containing crystal violet were used for detecting the presence of streptococci. Portions of each semen sample were streaked on the surface of previously prepared plates. Bacterial growth which had developed after incubation for 48 to 96 hr. at 37° C. was observed and the Gram morphology was determined in all cases of growth resembling streptococci.

The predominant types of bacteria were isolated from incubated veal infusion blood agar plates which had been employed for plate counts as reported previously (1). Isolated colonies were picked from the incubated plates and streaked on the surface of veal infusion blood agar plates in order to obtain maximum growth upon initial transfer. After incubation at 37° C. the resultant bacterial growth was subcultured and classified according to morphology, Gram reaction and biochemical activity (4).

EXPERIMENTAL

Incidence of P. aeruginosa in semen and the relation to fertility. The approximate numbers of *P. aeruginosa* were determined in 165 ejaculates from 30 bulls at various levels of fertility. The bulls were divided into three groups

based on fertility level. The fertility data were based on the percentage of cows which did not return for service within 90 to 120 days following the last insemination. The inseminations were made during the 4-month period in which the bacterial examination of the samples was conducted.

TABLE 1

The incidence of Pseudomonas aeruginosa in semen from bulls at various fertility levels

Level of fertility	Number of bulls	Number of ejaculates	Ejaculates containing		
			< 2/ml.	At least 2 but < 1,000/ml.	At least 1,000/ml.
Fertile (60-82) ^a	18	79	52	14	13
Relatively infertile (34-59)	10	76	49	15	12
Infertile ^{ab}	2	10		2	8
All levels	30	165	101	31	33

^a Per cent 90- to 120-day non-returns.

^b Semen not satisfactory for breeding.

As shown in table 1 the presence of *P. aeruginosa* was confirmed in numbers of at least two per ml. in 64 or about 39 per cent of the 165 ejaculates examined. Eighteen of the 30 bulls yielded at least one ejaculate in which *P. aeruginosa* was confirmed. However, the species was confirmed in all ejaculates examined from only 5 of the 18 bulls. Thus, it appears that, although certain bulls may be classified as "shedders" of *P. aeruginosa*, all ejaculates obtained from these bulls may not invariably contain the organism. Considerable variation in numbers of organisms also was noted between the various ejaculates obtained from the same bull.

Table 1 also shows that the incidence of *P. aeruginosa* was about equal in the fertile and relatively infertile groups of bulls. The organisms were confirmed in all ejaculates from three bulls in the fertile group and from the two bulls comprising the infertile group. The latter two bulls consistently produced semen of unsatisfactory quality for use in artificial breeding. The semen samples were characterized by having a low percentage of motile spermatozoa and a high percentage of morphologically abnormal cells. From these data it appears that although the presence of *P. aeruginosa* even in relatively large numbers is not related to level of fertility, its presence may be associated with individual cases of infertility. Additional data are needed as the fertilizing capacity of any particular semen sample still may be influenced by the presence of large numbers of *P. aeruginosa*.

Incidence of organisms of the coliform group. A total of 209 ejaculates from 35 bulls was examined for the presence of coliform organisms. Table 2 shows that only 24 or about 11 per cent of the ejaculates were found to contain at least 10 coliform organisms per ml. The organisms were found in semen from only 12 bulls and only one of the bulls was found to consistently "shed"

the organisms in all ejaculates examined. As shown in table 2, the organisms were not present in particularly large numbers, since only four ejaculates contained as many as 1,000 organisms per ml. In this study more than one ejaculate frequently was obtained from a single bull on a collection day. There were 109 first, 96 second and 4 third ejaculates collected. The incidence of coliform-positive ejaculates was 13 first, 9 second and 2 third ejaculates.

TABLE 2

The incidence of coliform organisms in semen from bulls at various fertility levels

Level of fertility	Number of bulls	Number of ejaculates	Ejaculates containing			
			< 10/ml.	At least 10 but < 100/ml.	At least 100 but < 1,000/ml.	At least 1,000/ml.
Fertile (60-82) ^a	20	92	78	5	8	1
Relatively infertile (34-59)	13	104	97	4	1	2
Infertile ^b	2	13	10	0	2	1
All levels	35	209	185	9	11	4

^a Per cent 90- to 120-day non-returns.

^b Semen considered unsatisfactory for breeding.

Table 2 also shows that the greatest percentage of positive ejaculates was found in the infertile group of bulls and that the percentage of positive ejaculates was greater in the fertile group than in the relatively infertile group. Together with the relatively low incidence of coliform organisms, it appears that there is no significant relation between the presence of coliform organisms in semen and fertility. Nevertheless, their presence definitely was associated with a high bacterial count. The average plate count of 202 of the 209 ejaculates examined for coliform organisms was 200,000 bacteria per ml. The 24 coliform-positive ejaculates averaged 1,400,000 bacterial per ml., as compared to only 91,000 per ml. for the 178 coliform-negative ejaculates.

Thus, coliform organisms were found only occasionally in semen from bulls at various levels of fertility and in relatively small numbers. These results suggest that the presence of organisms of the coliform group is the result of fecal contamination of the semen at the time of collection rather than elimination of the organisms from the genital tract of the bull along with the semen. However, data more recently gathered by this laboratory indicate that semen collected from one bull consistently contains coliform organisms.

Incidence of streptococci in semen. Two hundred and twelve ejaculates from 36 bulls were examined for the presence of streptococci. No evidence of these organisms was found in any of the semen samples examined. This confirms the findings of Gunsalus *et al.* (10).

The predominant types of bacteria in bull semen and their relationship to fertility. The incidence of bacteria in the 19 ejaculates from 8 bulls at various levels of fertility is presented in table 3. Sixty-one per cent of the 457 isolations were gram-positive rods. Species of *Corynebacterium* constituted the

major portion of the flora of this group of bacteria. This genus also represented about 44 per cent of the isolations from the highly fertile group and about 30 per cent of those from the less fertile group. Thus it appeared that the presence of this group was common to nearly all samples of semen and occurred in greatest numbers in semen from the more highly fertile bulls.

TABLE 3
Predominant types of bacteria in bull semen

Level of fertility	Bull	% 90- to 120-day non-returns	No. of ejaculates	No. of cultures	Per cent of cultures classified as			
					Gram-positive rods	Gram-positive micrococci	Gram-negative rods	Others
Fertile (60-69)*	H-2	69	2	85	61	26	4	9
	G-7	68	2	34	65	12	15	8
	H-1	65	2	49	76	6		18
	Average or total	67	6	168	66	17	5	12
Relatively infertile (40-59)	G-13	54	1	27	45	48	7	
	H-10	53	3	68	60	32	6	2
	G-5	51	3	67	74	10	10	6
	H-14	45	2	48	31	17	52	
	H-15	40	4	79	67	14	18	1
	Average or total	48	13	289	59	21	18	2
Average or total		61	19	457	61	20	13	6

* Per cent 90- to 120-day non-returns.

Although most members of this genus are considered to be non-pathogenic, certain members of the group possess varying degrees of virulence and have been associated with numerous pyogenic infections. However, the cultural characteristics of the majority of these organisms resembled those of the saprophytic forms and these organisms undoubtedly belong to the group commonly known as diphtheroids. The presence of a few of the more pathogenic types was suggested by reactions on blood agar and litmus milk.

Gram-positive micrococci were found in all but one of the semen samples. A greater percentage of these organisms were isolated from the relatively infertile bulls than from the fertile bulls. Many of these organisms appeared as the more inert forms of micrococci. However, certain types were encountered in semen from one relatively infertile bull, H-10, which appeared to be more virulent in nature. These cultures produced a deep golden pigment, hemolyzed red blood cells and were extremely active on the culture media employed. Thus it appeared that certain pyogenic forms were contained within the group.

Included in the group of gram-negative rods were species of the genera *Pseudomonas*, *Flavobacterium* and *Alcaligenes*. Coliform organisms were not encountered in this particular phase of the study. *P. aeruginosa* was encountered only in semen from two relatively infertile bulls, H-14 and H-15. Although this species was not found during this phase of the experiment in semen from any of the fertile bulls, the presence of the organism was confirmed in two ejaculates from one fertile bull, H-2, when asparagine broth was employed as a

selective medium. In general the organisms of the group appeared to be non-pathogenic, avirulent in nature and consisted of types commonly present on such materials as bedding, soil, feces, etc. However, on the basis of these data, which show a greater percentage of gram-negative rods in semen from relatively infertile bulls than from fertile bulls it is possible that members of this group may be associated with infertility. Further investigations are needed to definitely establish this relationship.

A few other types of organisms were encountered that did not fall into the above classification. About 5 per cent of all the isolates were members of *Actinomycetaceae* and a few false yeasts also were isolated. Due to the low incidence of these organisms it is believed that their presence in semen had no particular relation to fertility.

SUMMARY

By the use of special selective media the incidence of *Pseudomonas aeruginosa*, coliform organisms and streptococci was determined in undiluted semen from bulls used for artificial breeding. In addition, the predominant types of bacteria present in semen were isolated and classified according to morphological and biochemical characteristics.

1. *P. aeruginosa* was confirmed in semen samples from both fertile and relatively infertile bulls. The organism was confirmed in all ejaculates examined from three fertile and two infertile bulls. Even in numbers of at least 1,000 per ml. the presence of the organisms was not indicative of level of fertility. However, the consistent presence of the organism in semen of the infertile bulls indicated that it may be associated with individual cases of infertility.

2. Coliform organisms were found only occasionally in semen from bulls at various levels of fertility. The presence of the group was associated with high plate counts of semen and may represent fecal contamination of the sample at the time of collection.

3. Members of the genus *Streptococcus* were not characteristic of the seminal flora.

4. Gram-positive rods, especially diphtheroids, were found to be predominant in the flora of bull semen. These organisms comprised a greater portion of the flora of fertile bulls than that of relatively infertile bulls.

5. Gram-positive micrococci were found next in frequency to the diphtheroidal flora. It perhaps was significant that the proportion of the flora represented by these forms was slightly greater in the case of bulls of low fertility.

6. Although the gram-negative rods encountered appeared as non-pathogenic types commonly present in nature, a greater percentage was found in semen from the relatively infertile bulls and the presence of certain of these organisms in semen may be associated with infertility.

REFERENCES

- (1) ALMQUIST, J. O., PRINCE, P. W., AND REID, J. J. Bacteriological Studies of Bovine Semen. I. Numbers of Bacteria and the Relation to Fertility. *J. Dairy. Sci.*, 32: 543-548. 1949.

- (2) American Public Health Association. *Standard Methods for the Examination of Water and Sewage*. 9th ed. 1946.
- (3) BEAVER, D. C., BOYD, W. L., AND FITCH, C. P. A Contribution to the Bacteriology and Pathology of Sterility in Cows, with Report of Nineteen Cases. *J. Am. Vet. Med. Assoc.*, **61**: 469-502. 1922.
- (4) BERGEY, D. H. *Bergey's Manual of Determinative Bacteriology*. 6th ed. The Williams and Wilkins Co., Baltimore, Maryland. 1948.
- (5) BORG, H. Enzootische Sterilität by het Rund. (Enzootic Sterility in Cattle) *Trydschr Diergeneesk.*, **70**: 40-54. 1943. (Biological Abstracts, **21**: 12214. 1947.)
- (6) EDMONDSON, J. E., TALLMAN, K. L., AND HERMAN, H. A. A Study of the Types of Bacteria in Bovine Semen and Their Effect Upon Motility (Abstract). *J. Dairy Sci.*, **31**: 681. 1948.
- (7) FITCH, C. P. A Preliminary Study of the Pathology and Bacteriology of Ovaritis in Cattle. Report of the New York State Veterinary College, p. 199. 1915-16.
- (8) GUNSALUS, I. C., CAMPBELL, J. J., BECK, G. H., AND SALISBURY, G. W. The Bacteriology of Bull Semen. II. The Effect of Bacteria upon Rapid Tests for Semen Quality. *J. Dairy Sci.*, **27**: 357-364. 1944.
- (9) GUNSALUS, I. C., SALISBURY, G. W., AND WILLETT, E. L. The Bacteriology of Bull Semen. *J. Dairy Sci.*, **24**: 911-919. 1941.
- (10) HATZIOLOS, B. Untersuchungen über Konservierung von Bullensperma zum Zwecke der künstlichen Besamung. *Ztschr. f. Zucht.*, **36 B**: 199-254. 1937. (Citation from Gunsalus *et al.* Ref. no. 9.)
- (11) KRZYSZKOWSKY, K. N., AND PAWLOW, G. N. Beiträge zur Biologie der Spermatozoen. *Ztschr. f. Tierzucht u. Zuchtungsbiol.*, **10**: 257-283. 1927. (Citation from Gunsalus *et al.* Ref. no. 9.)
- (12) OGNIANOV, A. La Carica Batterica nello Sperma Bovino Ottenuto Strumentalmente nella Pratica della Fecondazione Artificiale. (The Bacterial Content of Bovine Semen Collected for Artificial Insemination.) Istituto Sperimentale Italiano "L. Spallanzani" per la Fecondazione Artificiale (Milano), pp. 48-49. 1942. (Animal Breeding Abstracts, **15**: 86-87. 1947.)
- (13) POUNDEN, W. D., FERGUSON, L. C., KNOOP, L. C., AND KRAUSS, W. E. A Further Report on Staphylococci Abortions in a Dairy Herd. *J. Am. Vet. Med. Assoc.*, **111**: 376-378. 1947.
- (14) Report of the Chief of the Bureau of Animal Industry. U. S. Dept. Agr., p. 5. 1942.
- (15) ROEMMELE, O. Biologische und physiologische Untersuchungen am Sperma und am Scheidensekret des Rindes im Hinblick auf die künstliche Besamung. *Zool. Jahrb. Abt. f. Allg. Zool. u. Physiol.*, **44**: 85-148. 1927. (Citation from Gunsalus *et al.* Ref. no. 9.)
- (16) UCHIGAKI, S. Biological Research of Sterility. I. Influence of Various Bacillus Toxins on Spermatozoa. *Japanese Jour. Obstetr. and Gynecol.*, **10**: 5-19. 1927. (Biological Abstracts, **4**: 15364. 1930.)
- (17) U'DALL, D. H., CHSHING, E. R., AND FINCHER, M. G. Vital Statistics of Diseases of the Genital Organs of Cows. *Cornell Vet.*, **15**: 121-136. 1925.
- (18) WEBSTER, W. M. Bovine Sterility in New Zealand. *Austral. Vet. J.*, **8**: 199-222. 1932.
- (19) WILLIAMS, W. W. Technique of Collecting Semen for Laboratory Examination with Review of Several Diseased Bulls. *Cornell Vet.*, **10**: 87-94. 1920.

THE FERTILITY OF BOVINE SEMEN COOLED WITH AND WITHOUT THE ADDITION OF CITRATE-SULFANILAMIDE-YOLK EXTENDER¹

R. H. FOOTE AND R. W. BRATTON

Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry, Cornell University, Ithaca, New York

In artificial breeding the procedure of adding extender to semen prior to cooling is now, with several exceptions, routine practice. However, no experimental data have been reported indicating the relative value, as measured in terms of fertility, of cooling bovine semen with or without the addition of extender.

With the exception of Steensma (15), most investigators (2, 3, 4, 7, 8, 12) have found that rapid cooling of unextended bovine semen decreases the survival time of spermatozoa and, consequently, they have recommended that semen be cooled slowly. Easley, Mayer, and Bogart (4) have shown that rates of cooling which decreased the percentage of spermatozoa surviving in unextended semen were without harmful effects when the semen was extended 1:3 with phosphate-yolk prior to cooling. However, none of the cooling rates which they employed was particularly rapid. Phillips and Lardy (13) noted that yolk-buffered semen could be cooled without "undue care". Later Lasley, Easley and Bogart (9), Lasley and Mayer (10), and Mayer and Lasley (11) isolated a factor in egg yolk which increased the resistance of spermatozoa to "cold shock".

Using rapid cooling procedures (30° C. decrease/min.) Willett and Salisbury (16) reported that the motility of spermatozoa in semen extended 1:4 with phosphate-yolk or citrate-yolk as well as the unextended semen was affected adversely. Storage data recently obtained in this laboratory (5, 6) on semen extended at rates of 1:12 and 1:100 before cooling have indicated that, irrespective of the rate of cooling, partial or complete extension in buffered yolk before rather than after cooling was the more effective in maintaining spermatozoan livability. Recently Anderson and Seath (1) have shown that a delay in cooling, and especially a delay in extending semen, results in semen with poorer keeping qualities. This work suggests that semen should be extended immediately. However, in all of these reports the value of the procedures recommended has not been measured in terms of fertility. In view of this fact the experiment reported herein was designed to compare, by using split ejaculates, the fertility of pre-extended semen (semen cooled in citrate-sulfanilamide-yolk extender) with post-extended semen (semen cooled without the addition of extender).

EXPERIMENTAL

Design. The pre-extended and post-extended portions of each ejaculate were shipped to alternate groups of ten technicians each. Thus, any one ejaculate

Received for publication June 11, 1949.

¹ In view of the improved livability of spermatozoa in present day media the authors consider the word "extender" more appropriate than "diluter" for describing these media, and therefore have adopted the word "extender" in this report.

was shipped to 20 technicians or multiples of 20 technicians. To insure that all technicians would have approximately equal opportunities to use each treatment, those groups receiving pre-extended semen during the first 8 days received post-extended semen during the last 8 days of the experiment.

Procedure. Immediately following collection, each ejaculate was divided into two equal portions. One portion of semen at 30° C. then was extended on the average of 1:4 with citrate-sulfanilamide-yolk extender² while the other portion was left unextended. A sterile thermometer was placed in each portion to observe the rates of cooling. Each portion then was placed in a tumbler filled with water at 30° C., and the tumblers placed in a walk-in-type refrigerator maintained at 5° C. After 20 minutes the tubes containing semen were removed from the tumblers and allowed to stand in the cold room air for 55 minutes before the extension to final volume was made. This method of water and air cooling was standard procedure for the New York Artificial Breeders' Cooperative, Inc. Following extension to final volume, 3 ml. sub-samples, which filled to capacity the test tubes used, were taken from each treatment of each ejaculate for the purpose of observing microscopically the motility of the spermatozoa during storage at 5° C. These observations were made after 3, 24, 48, 72 and 96 hr. of storage.

Sixty-four ejaculates from 31 Holstein bulls owned by the New York Artificial Breeders' Cooperative, Inc. were used for insemination. These ejaculates represented nearly all of the semen collected from these bulls during the experimental period, only those ejaculates containing few spermatozoa or otherwise decidedly inferior being discarded. The semen samples initially contained an average of $1,647 \times 10^6$ total spermatozoa per ml., of which 70 per cent were estimated to be motile. All ejaculates were extended so as to contain a minimum of approximately 15 million (range 10–25 million) live spermatozoa per ml. of extended semen. The average number for the experiment was approximately 17 million per ml.

Measurement of Fertility. The measurement of fertility was based on the per cent of first and second service cows not returning to service within 60 to 90 days after insemination, these inseminations being performed by the regularly employed technicians affiliated with the New York Artificial Breeders' Cooperative, Inc. The per cent non-returns for each treatment of each ejaculate (treatment \times ejaculate sub-group) was considered as the experimental unit. The statistical significance of the differences between treatment means for the per cent non-returns for first, second, and the combined first and second service cows were tested by analysis of variance (14).

RESULTS

The average cooling rates for the pre-extended and post-extended semen are shown graphically in figure 1. Both portions of semen cooled slowly and at

² The citrate-sulfanilamide-yolk extender consisted of equal parts of fresh egg yolk and a buffer containing 3.6% sodium citrate dihydrate and 0.6% sulfanilamide.

essentially the same rate while in the tumblers of water. When placed in air at 5° C. the pre-extended semen samples, because of their larger volumes, cooled at a slightly slower rate than did the post-extended samples. Cooling was accomplished in 75 min.

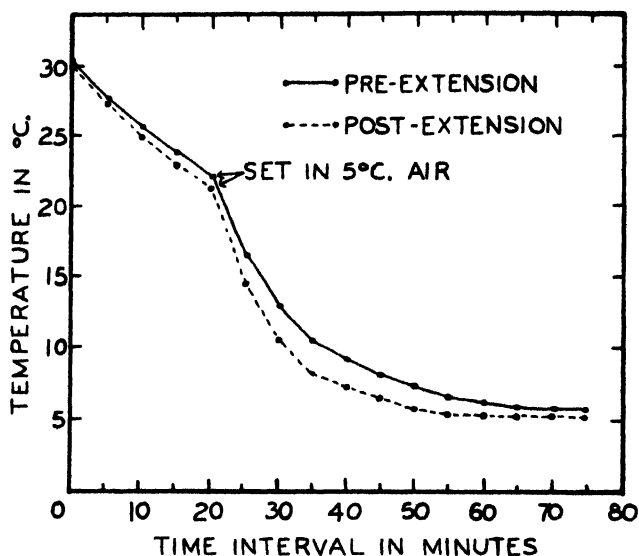


FIG. 1. Comparison of the rates at which pre-extended and post-extended semen were cooled (average of 64 semen samples).

The average per cent of motile spermatozoa and the average rate of progressive motility in the sub-samples after 3, 24, 48, 72, and 96 hr. of storage at 5° C. are summarized in table 1. Even with slow cooling, the proportion of motile

TABLE 1
Average of motility estimations of pre-extended and post-extended semen during storage at 5° C.
(64 ejaculates used for insemination)

Storage time (hr.)	Pre-extension		Post-extension	
	% Motile	Rate ^a	% Motile	Rate ^a
3	63	2.4	48	2.0
24	56	2.0	41	1.6
48	51	1.7	34	1.3
72	47	1.5	30	1.0
96	42	1.3	23	0.9

^a—Arbitrary scale indicative of progressive motility with 4.0 as a maximum and 0 as no motility.

spermatozoa was much higher in the pre-extended semen than it was in the post-extended semen. That the detrimental effect was an immediate one is evidenced

by the motility estimations made 3 hr. after the semen was collected. Routine semen examinations made by the laboratory personnel of the New York Artificial Breeders' Cooperative, Inc. and the technicians affiliated with this organization were in agreement with the data in table 1.

The semen shipped in this experiment was used to inseminate 5,769 first-service and 2,749 second-service cows. The fertility results based on 60- to 90-day non-returns to service are shown in table 2. The fertility level of the pre-

TABLE 2
*Fertility of pre-extended and post-extended semen
(based on 60- to 90-day non-returns to service)*

	Pre-extension		Post-extension		Difference between treatment means
	No. serv.	% ^a N. R.	No. serv.	% ^a N. R.	
1st serv. cows	2880	61.8	2889	55.0	6.8 ^b
2nd serv. cows	1404	55.1	1345	48.5	6.6 ^c
1st and 2nd serv. cows	4284	59.3	4234	52.8	6.5 ^b

^a = % N. R. are the means of the per cent non-returns of each treatment \times ejaculate sub-group which was used in the analysis of variance.

^b = Significant at the 1% level of probability.

^c = Significant at the 5% level of probability.

extended semen was 6.5 percentage units higher than that of the post-extended semen. Not only is this difference highly significant statistically but it demonstrates experimentally the practical importance of placing spermatozoa in extender before cooling them.

Since presumably there were fewer motile spermatozoa in the post-extended samples at the time they were used for insemination, the authors were interested in determining whether or not the observed difference in fertility could be partly accounted for by the differences in the estimated number of motile spermatozoa per insemination. By covariance analysis using the number of motile spermatozoa inseminated as the independent variate (X) and the per cent non-returns recorded for each treatment \times ejaculate sub-group as the dependent variate (Y) it was found that within this experiment the higher per cent non-returns obtained with the pre-extended semen could not be accounted for on the basis of more motile spermatozoa per insemination. Whether or not this increase in fertility should be attributed to the "cold shock" factor of egg yolk or whether it reflects immediate extension *per se*, as suggested by Anderson and Seath (1), has not been elucidated.

SUMMARY

Sixty-four ejaculates of bovine semen were divided and cooled from 30° C. to 5° C. in 75 minutes with and without the addition of citrate-sulfanilamide-yolk extender prior to cooling.

Based on 60- to 90-day non-returns to 8,518 first and second service cows, the fertility level of the pre-extended semen (semen cooled in extender) was 59.3

per cent and that of the post-extended semen (cooled without extender), 52.8 per cent. The difference between treatments of 6.5 percentage units was highly significant statistically.

Motility estimates made after 3, 24, 48, 72 and 96 hr. of storage indicated that the samples cooled without extender had a definitely lower percentage of motile spermatozoa. However, by using covariance analysis the higher per cent non-returns for the pre-extended semen could not be accounted for on the basis of more motile spermatozoa per insemination.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of the management of and the technicians associated with the New York Artificial Breeders' Cooperative, Inc. in supplying and processing the semen and in performing the inseminations, and the assistance of Mrs. Delma Muller in summarizing the data.

REFERENCES

- (1) ANDERSON, H. W., AND SEATH, D. M. Effect of Delay in Diluting and Cooling on Keeping Quality of Bull Semen. *J. Dairy Sci.*, 31: 551-555. 1948.
- (2) BIRILLO, I. M., AND PUHALJISKII, L. H. Voprosy dlitel'nogo schranenija spermy byka i farana. (Problems of Prolonged Storage of Bull and Ram Spermatozoa.) *Problemy Zhivotovod.*, 10: 24-30. 1936. (*Animal Breeding Abstracts*, 5: 219-220. 1937. Original not seen.)
- (3) DAVIS, H. P. Some Factors Affecting Artificial Insemination in Cattle. *Proc. Am. Soc. Animal Production*, 31: 246. 1938.
- (4) EASLEY, G. T., MAYER, D. T., AND BOGART, R. Influence of Diluters, Rate of Cooling, and Storage Temperatures on Survival of Bull Sperm. *Am. J. Vet. Research*, 3: 358-363. 1942.
- (5) FOOTE, R. H., AND BRATTON, R. W. The Fertility of Bovine Semen Cooled With and Without the Addition of Citrate-Sulfanilamide-Yolk Extender. *J. Dairy Sci.*, 32: 723. 1949.
- (6) FOOTE, R. H. Unpublished data. Cornell University. 1948.
- (7) GLADCINOVA, E. F. Vlijanie rezkih poniženij temperatury na pereževanie spermatozoidov. (The Influence of Marked Drops in Temperature on the Survival of Spermatozoa.) *Uspekhi Zooteh. Nauk.*, 4: 56-64. 1937. (*Animal Breeding Abstracts*, 7: 373. 1939. Original not seen.)
- (8) HERMAN, H. A., AND RAGSDALE, A. C. Artificial Insemination of Dairy Cows. *Mo. Agr. Expt. Sta. Bull.* 407. 1939.
- (9) LASLEY, J. F., EASLEY, G. T., AND BOGART, R. Some Factors Influencing the Resistance of Bull Semen to Unfavorable Environmental Conditions. *J. Animal Sci.*, 1: 79. 1942.
- (10) LASLEY, J. F., AND MAYER, D. T. A Variable Physiological Factor Necessary for the Survival of Bull Spermatozoa. *J. Animal Sci.*, 3: 129-135. 1944.
- (11) MAYER, D. T., AND LASLEY, J. F. The Factor in Egg Yolk Affecting the Resistance, Storage Potentialities, and Fertilizing Capacity of Mammalian Spermatozoa. *J. Animal Sci.*, 4: 261-269. 1945.
- (12) MOROZOV, V. A. Primenenie nizkikh temperatur dlja hraniénija spermy bjika. (The Application of Low Temperatures for Storage of Bull Sperm.) *Vestn. sel'skokhoz. Nauk. Zivotn.*, No. 1: 139-147. 1940. (*Animal Breeding Abstracts*, 10: 99. 1942. Original not seen.)

- (13) PHILLIPS, P. H., AND LARDY, H. A. A Yolk-buffer Pabulum for the Preservation of Bull Semen. *J. Dairy Sci.*, 23: 399-404. 1940.
- (14) SNEDECOR, G. W. *Statistical Methods*. 4th Ed. Iowa State College Press, Ames. 1946.
- (15) STEENSMA, R. Storage of Ram and Bull Semen. Master's Thesis, University of Missouri, 1938. (Cited in Mo. Agr. Expt. Sta. Res. Bull. 326. 1941.)
- (16) WILLETT, E. L. AND SALISBURY, G. W. The Effect of Various Diluters, Cooling Rate, Temperature of Storage, and Some Other Factors, on the Livability of Spermatozoa in Stored Samples of Bull Semen. Cornell University Memoir 249. 1942.

LIBERATION OF FATTY ACIDS DURING MAKING AND RIPENING OF CHEDDAR CHEESE¹

MERLIN H. PETERSON AND MARVIN J. JOHNSON

Department of Biochemistry

AND

WALTER V. PRICE

Department of Dairy Industry, Univ. of Wisconsin, Madison

The determination of the chemical changes occurring in the fat of Cheddar cheese during ripening has been the object of numerous investigations. In 1910 Suzuki *et al.* (17) reported the results of partitioning by the Duclaux technique the volatile fatty acids obtained from steam distillation of acidified suspensions of Cheddar cheese. Their results are subject to question since the Duclaux technique is inadequate for the identification and analysis of complex mixtures of fatty acids such as those found in Cheddar cheese.

Since this early study most investigators have measured the free fatty acids of Cheddar cheese as a group and not as individual components. Among the methods most often used are the direct determination of cheese fat acidities (1, 9, 10) and steam or direct distillation of acidified cheese suspensions (5, 8, 11) or extracts (6, 7, 16), followed by distillate titrations. These methods do not give accurate qualitative or quantitative data on the nature and levels of individual free fatty acids present in Cheddar cheese. Undoubtedly these limitations with regard to Cheddar cheese free fatty acid methods have contributed to the failure of many investigators to find a relationship between Cheddar cheese flavor and free fatty acid content (1, 2, 3, 7, 10, 15).

In a previous publication (14) from this laboratory, a rapid partition chromatographic method was reported for the quantitative estimation of formic, acetic, propionic, *n*-butyric, caproic, caprylic and capric acids in biological materials. These fatty acids of short and intermediate chain length are very rich in characteristic flavor and odor and are considered to be important in Cheddar cheese flavor. Naturally-occurring fatty acids having more than ten carbon atoms are quite bland in flavor and odor and consequently are believed to play little or no part in cheese flavor.

The purpose of the present investigation is to determine the nature and levels of individual free fatty acids present during the making and ripening of raw and pasteurized Cheddar cheeses from the same milk. The application of the partition chromatographic method to the estimation of free fatty acids in Cheddar cheese also is described.

METHODS

Cheesemaking procedure. Two 460-lb. identical lots of mixed, raw, whole milk from the University dairy were used. After one lot had been pasteurized

Received for publication June 13, 1949.

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by a grant from the National Cheese Institute.

at 145° F. for 30 minutes, both lots were placed in vats and subjected to identical subsequent treatment. From each lot two 22-lb. non-colored cheeses were made by an experienced cheesemaker according to standard procedures. The green cheeses were kept at 68° F. for 2 to 3 days, then transferred to a cold room (40 to 45° F.) for ripening.

Free fatty acids. The method for the determination of free fatty acids in biological materials previously reported by the present authors (14) was used directly for all analyses of free fatty acids in cheese milk, rennet, starter culture samples and cheeses during the first 6 months of their ripening periods.

In the application of the method to Cheddar cheese older than 6 months or artificially colored, a slight modification of the sample preparation procedure as outlined in the original report (14) was necessary. Substances present carried through the original procedure into the benzene extracts used as the sample on the benzene-33N sulfuric acid macro separation column. Their effect on this column was a slow displacement of non-mobile phase. The causative substance in young artificially colored Cheddar cheese was subsequently found to be bixin added as coloring material; that of aged non-colored cheese has not been identified. It was found that both types of substances can be removed successfully from the benzene extracts by the use of an additional macro separation column during the original sample preparation procedure.

The modified procedure is identical with the original procedure prior to the step in which the benzene-33N sulfuric acid macro separation column is used. In the modified procedure this column is developed until bixin and bixin-like substances have just reached the column base but not passed into the effluent, as indicated by the erratically-progressing brown zones they form on the column. The benzene effluent, approximately 100 ml. in volume, contains not only all the capric and higher acids but also small amounts of the caprylic and lower fatty acids. The fatty acids are removed from the benzene effluent by several extractions with small volumes of dilute sodium hydroxide. The aqueous solution is concentrated, if necessary, to 100 ml. or less, brought to pH 2 with 5 per cent sulfuric acid, and extracted with three 10-ml. portions of thiophene-free benzene. The residual aqueous solution is combined with the aqueous residue of the original benzene extraction and set aside. The last traces of aqueous phase are separated from the benzene centrifugally and washed once with 5 ml. of fresh benzene. The benzene solution is added without previous equilibration with sulfuric acid to a second benzene-33N sulfuric acid macro separation column in which the quantitative separation of the capric and higher acids from the caprylic and lower fatty acids is accomplished without interference from bixin and bixin-like substances.

The contents of the two macro separation columns, including packing, are combined with the aqueous residues from the two benzene extractions. The benzene is removed from the resulting suspension by distillation at pH 8.5. After cooling, the residue is brought to pH 2 with sulfuric acid and the volatile acids are distilled by the magnesium sulfate method described in the original procedure. The bixin and bixin-like substances are non-volatile and remain in the distillation

flask residue which is discarded. From this point on, the distillate which contains all of the lower fatty acids (caprylic and below) of the original sample and the neutralized benzene effluent of the second macro separation column containing the higher fatty acids (capric and higher) subsequently are prepared and chromatographically analyzed exactly as outlined in the original procedure.

FREE FATTY ACID CONTENT OF CHEDDAR CHEESE DURING MAKING AND RIPENING

Free fatty acid content of raw and pasteurized Cheddar cheese after making.

In figure 1 the average levels of the individual free fatty acids of two series of

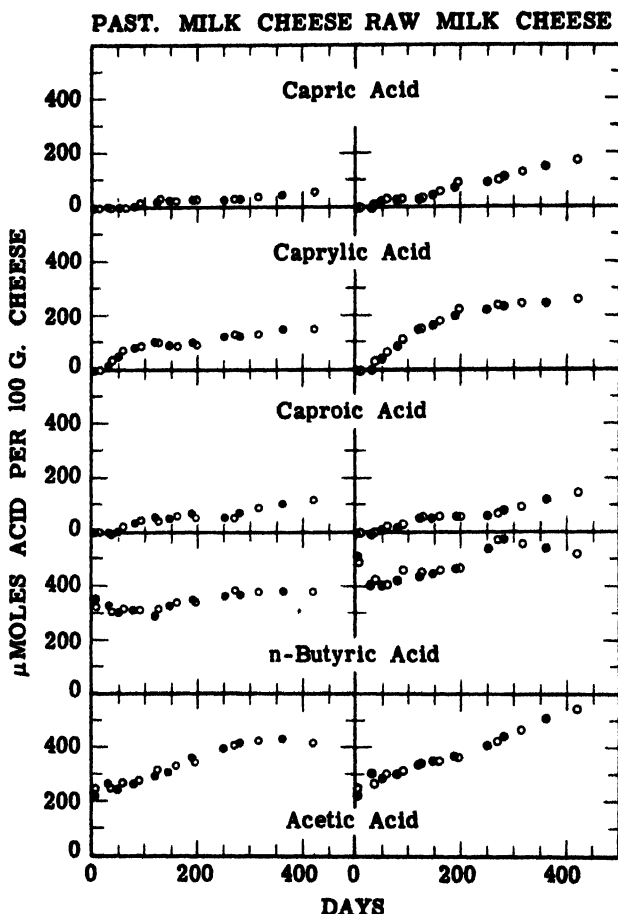


FIG. 1. Free fatty acids in Cheddar cheese during a 420-day ripening period. (The points represent average values for two series each with four pairs of cheese. Series 1 data ●; series 2 data ○.)

pairs of pasteurized milk cheeses and raw milk cheeses made by the procedure described under "Methods" are given. Individual free fatty acids isolated

during the partition chromatographic analytical procedure were checked for identity in two ways. The first of these was their zone positions in column effluents. The second consisted of rechromatographing an isolated fatty acid on a suitable column after adding an equivalent amount of an acid presumably identical. If only one zone was formed and quantitative recovery of the added known fatty acid from that zone was obtained, the two acids were considered identical. By the use of these criteria only straight-chain fatty acids having an even number of carbon atoms, such as are shown in figure 1, have been detected in Cheddar cheese; fatty acids having an odd number of carbon atoms are either absent or present at very low levels.

As may be seen in figure 1, 420-day old raw milk cheese has considerably higher levels of caprylic and capric acids and moderately higher levels of *n*-butyric and acetic acids than corresponding pasteurized milk cheese. Caproic acid levels for both types of cheese are approximately equivalent. The higher flavor generally developed in aged raw milk Cheddar cheese over corresponding pasteurized milk cheese is believed due in part to the higher levels of free volatile fatty acids in the raw milk cheese.

Figure 1 also shows that caproic, caprylic and capric acids are absent in both raw and pasteurized milk cheeses during the first 30 days of ripening, while acetic acid is present at approximately identical high levels in both types of cheese. *n*-Butyric acid also is found at high levels in both types of cheese during this period, although at a slightly higher concentration in the raw milk cheese.

As the two types of cheese age, acetic acid levels increase for both through 300 days of ripening. Since lactose is absent from Cheddar cheese after the first few days of ripening, most of the acetic acid produced during the ripening period is probably due to lactate and protein decomposition by the cheese bacterial flora.

In a previous report (13) the present authors demonstrated that lipases, active at the pH of ripening Cheddar cheese, make their appearance in young Cheddar cheese after 5 to 10 days of ripening. It was suggested that these lipases may represent intracellular lipases of lactic acid bacteria liberated by bacterial autolysis. It is further suggested that part of the free *n*-butyric acid and all of the free caproic, caprylic, and capric acids shown above to be present in 420-day-old raw and pasteurized milk Cheddar cheese are the result of the action of these liberated intracellular bacterial lipases on the milk fat of the cheese. The differences in amounts of these fatty acids in raw milk cheese and corresponding pasteurized milk cheese possibly are due to a large reduction in numbers by pasteurization of bacterial species in milk capable of liberating intracellular lipases through autolysis.

Free fatty acid content of raw and pasteurized Cheddar cheese during making. Upon completion of the studies on the individual free fatty acids of Cheddar cheese after making, it was found that no information on the sources of the high levels of free acetic and *n*-butyric acids present in both raw and pasteurized milk cheese at the start of the ripening period (figure 1) could be found in the data. Consequently, detailed studies on levels of individual free fatty acids present in

raw and pasteurized milk cheese during making and the early stages of ripening were carried out. The results of these studies are given in figure 2 which shows the average levels of the individual free fatty acids of a series of pairs of pasteurized milk cheeses and raw milk cheeses other than those used in the ripening studies.

At the start of making, only the acetic and *n*-butyric acids of the cheese milk are significant in amount. The levels of these acids present in both the raw and pasteurized milk lots at the time they are placed in the cheese vats are shown in

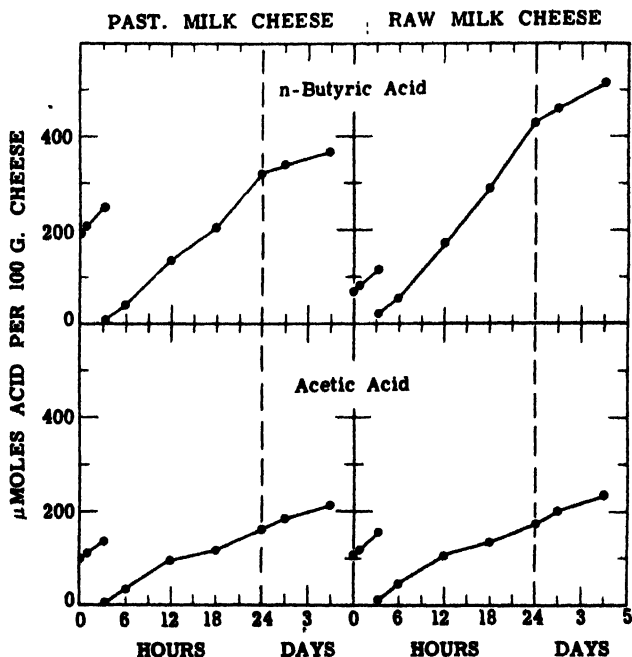


FIG. 2. Free fatty acids in Cheddar cheese during making and the early stages of ripening. (The points represent average values for four pairs of cheese. Breaks in the curves represent times of dipping (200 min.). Of the two fatty acid levels given at the dipping time, the upper represents the acid removed in the whey while the lower represents that remaining in the curd.)

figure 2. Since the free fatty acids contributed by the starter culture and rennet extract added during making are not significant, the small increases in acetic acid concentration during the first 200 min. of making undoubtedly are due to bacterial action on milk lactose while those of *n*-butyric acid are probably the result of rennet lipolytic activity (13) and bacterial activity. It will be noted that the acetic and *n*-butyric acids present in the whey-curd mixture for both types of cheese at the time of dipping (200 min.) can be almost completely accounted for in the whey after its removal from the cheese curd. The levels of the two acids in both raw and pasteurized milk curds increase rapidly during the ensuing 20-hr.

period until the levels of each present in cheese at the start of the ripening period are reached (figure 1). The formation of acetic acid during this period is very probably due in large part to bacterial action on milk lactose carried over into the cheese curd. The rapid rise in free *n*-butyric acid levels in the cheese curd immediately after dipping is not as easily explained. It does not arise from the milk lipase previously studied (12, 13) since this enzyme not only is inactivated in the pH range encountered in Cheddar cheesemaking prior to dipping, but also is destroyed during milk pasteurization (4). The complete absence of any type of lipolytic activity in Cheddar cheese between the time of dipping and the fifth day of the ripening period (13) suggests that the free *n*-butyric acid in Cheddar cheese at the start of ripening probably arises as a fermentation product.

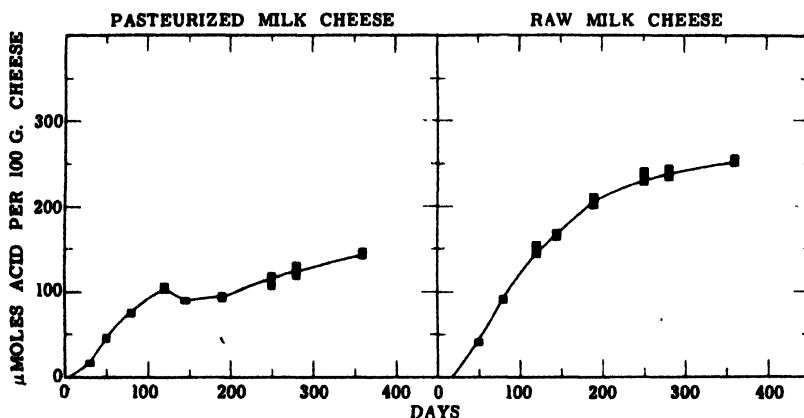


FIG. 3. Variation in free caprylic acid content of Cheddar cheeses of the same age. (The ranges of free caprylic acid content represent the individual acid levels found in the cheeses of Series 1 (figure 1). The intersections of the ranges with the curve show average values.)

Reproducibility of cheeses. The data of figure 1 represent averages of two series of four pairs of raw and pasteurized milk cheeses while that of figure 2 represent averages of a third series of four pairs of raw and pasteurized cheeses. The individual curves for each of the pairs of cheeses in each series are very similar to the average values presented for that series. The variation ranges of free caprylic acid levels at various stages of the four pairs of cheeses making up the first series used in figure 1 are presented in figure 3 and may be seen to be within reasonable limits.

SUMMARY

1. The content of individual free fatty acids of pairs of raw and pasteurized milk Cheddar cheeses has been determined by a chromatographic method at intervals during the making and ripening periods.

2. In both raw and pasteurized milk cheese during the first 30 days of ripening, caproic, caprylic, and capric acids are absent, while *n*-butyric acid is present at slightly lower levels than those of the same cheeses at 420 days. Acetic acid levels for both types of cheese are approximately one half those of the same cheeses at 420 days.

3. Raw milk cheese at 420 days has considerably higher levels of *n*-butyric and acetic acids than corresponding pasteurized milk cheese. Caproic acid levels for the two types are quite similar.

4. Of the free fatty acids contributed by the milk, rennet extract, and starter culture used during cheesemaking, only the acetic and *n*-butyric acids of the milk are significant in amount. There is, however, negligible carryover of these acids into the finished cheese, since they can be accounted for completely in the whey at the time of dipping.

5. The action of intracellular bacterial lipases on the cheese fat is believed to be responsible for part of the free *n*-butyric and all of the free caproic, caprylic, and capric acids present in aged raw and pasteurized milk Cheddar cheese.

REFERENCES

- (1) BABEL, F. J., AND HAMMER, B. W. Fat Degradation in Cheddar Cheese Made from Pasteurized Milk, Without and With Added Lipase. *J. Dairy Sci.*, **28**: 201-208. 1947.
- (2) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. The Flavor, Volatile Acidity, and Soluble Protein of Cheddar and Other Cheeses. *J. Dairy Sci.*, **30**: 165-174. 1947.
- (3) DAHLBERG, A. C., AND KOSIKOWSKY, F. V. Flavor Development in Cheddar Cheese. *Natl. Butter and Cheese J.*, **38**(8): 44. 1947.
- (4) HETRICK, J. H., AND TRACY, P. H. Effect of High-temperature Short-time Heat Treatments on Some Properties of Milk. II. Inactivation of the Lipase Enzyme. *J. Dairy Sci.*, **31**: 881-887. 1948.
- (5) HISCOX, E. R., AND HARRISON, J. Volatile Acids of Cheese. I. Retentive Power of Cheese and Its Constituents. *J. Dairy Research*, **9**: 215-226. 1938.
- (6) HISCOX, E. R., HARRISON, J., AND WOLF, J. Z. Volatile Acids of Cheese. II. Methods of Extraction. *J. Dairy Research*, **9**: 227-232. 1938.
- (7) HISCOX, E. R., HARRISON, J., AND WOLF, J. Z. Volatile Acids of Cheese. III. Application of the Extraction Method. *J. Dairy Research*, **12**: 155-169. 1941.
- (8) KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Rapid Direct-distillation Method for Determining the Volatile Fatty Acids of Cheese. *J. Dairy Sci.*, **29**: 861-871. 1946.
- (9) LANE, C. B., AND HAMMER, B. W. Studies on the Fat of Cheddar Cheese. *Iowa State Col. J. Sci.*, **13**: 149-155. 1939.
- (10) LANE, C. B., AND HAMMER, B. W. Bacteriology of Cheese. VI. Relationship of Fat Hydrolysis to the Ripening of Cheddar Cheese. *Iowa Agr. Expt. Sta. Bull.* **291**. 1941.
- (11) MARQUARDT, J. C., AND DAHLBERG, A. C. Determining Volatile Acids in Cheese. *Natl. Butter and Cheese J.*, **32**(8): 40-42. 1941.
- (12) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Determination of Milk Lipase. *J. Dairy Sci.*, **26**: 233-240. 1943.
- (13) PETERSON, M. H., JOHNSON, M. J., AND PRICE, W. V. Lipase Activity During Making and Ripening of Cheddar Cheese. *J. Dairy Sci.*, **31**: 39-46. 1948.
- (14) PETERSON, M. H., AND JOHNSON, M. J. The Estimation of Fatty Acids of Intermediate Chain Length by Partition Chromatography. *J. Biol. Chem.*, **174**: 775-789. 1948.

- (15) SHEURING, J. J., AND TUCKEY, S. L. A Study of the Changes Occurring in the Fat Constants of Milk Fat of Cheddar Cheese During the Ripening Process. *J. Dairy Sci.*, 30: 803-816. 1947.
- (16) SMILEY K. L., KOSIKOWSKY, F. V., AND DAHLBERG, A. C. A Simplified Extraction-distillation Method for the Determination of the Volatile Fatty Acids of Cheese. *J. Dairy Sci.*, 29: 307-316. 1946.
- (17) SUZUKI, S. K., HASTINGS, F. G., AND HAET, E. B. The Production of Volatile Fatty Acids and Esters in Cheddar Cheese and Their Relation to the Development of Flavor. *Wis. Agr. Expt. Sta. Bull.* 11. 1910.

PREPARTUM MILKING. I. THE EFFECT OF PREPARTUM MILKING ON SOME BLOOD CONSTITUENTS OF THE COW¹

H. D. EATON, R. E. JOHNSON, C. F. HELMBOLDT, A. A. SPIELMAN, L. D. MATTERSON, E. L. JUNGHERR, J. H. KRAMER AND R. J. SLATE

Animal Industries Department and Animal Diseases Department, Storrs Agricultural Experimental Station, Storrs, Connecticut

Prepartum milking has been proposed for reducing the incidence of milk fever and decreasing mammary and umbilical edema (4, 20). However, Smith and Blosser (16) have reported that in a herd of Jerseys, prepartum milking did not decrease the incidence of milk fever. This study was undertaken to determine quantitatively the effect of prepartum milking upon the changes occurring at parturition in total blood hemoglobin, total blood serum calcium, total blood serum inorganic phosphorous, and plasma carotene and vitamin A, and on mammary and umbilical edema. Secondly these factors were studied in relation to two dietary regimes.

EXPERIMENTAL

Animals. A total of 43 cows of the Ayrshire, Guernsey, Holstein and Jersey breeds in the University of Connecticut herd which calved from November, 1947, through December, 1948, were used in the experiment. They were divided into four experimental groups which were equalized insofar as possible in respect to breed, age, number of previous lactations, anticipated length of dry period, health, ancestry and previous dietary history. Groups 1-A and 1-B were not milked prepartum, Group 1-A receiving the basal ration alone, and 1-B the basal ration + 1 million USP units of vitamin A daily for 30 days prior to the calculated parturition date. Groups 2-A and 2-B were milked prepartum, Group 2-A receiving the basal ration and Group 2-B the basal ration plus the vitamin A supplement.

For 8 weeks prior to the calculated parturition date and for 3 days postpartum, all cows received the same basal ration fed on the basis of liveweight. This consisted per 100 lb. of liveweight of 1 lb. of U.S. No. 2 alfalfa hay, 3 lb. of well matured corn silage, and 1 lb. of a grain mixture consisting largely of cereal grains and containing approximately 13.5 per cent crude protein. The hay, silage and grain contained on an average 10.79, 1.98 and 0.16 mg. of carotene per pound, respectively, as determined by the method of Moore and Ely (10) as modified by Nelson *et al.* (11). The vitamin A supplement was shark liver oil² containing 25 per cent by weight of crude soybean lecithin. This oil contained an average of 53,000 USP units of vitamin A per g. as assayed spectrophotometrically against the USP Vitamin A reference standard (vitamin A acetate in cottonseed oil). On the fourth day postpartum, the cows were returned to the milking herd where they received hay and silage *ad libitum* and grain according to milk production.

Received for publication June 16, 1949

¹ This work was supported in part by the Big-Y-Foundation, Norwich, Conn. and Chas. M. Cox Co., Boston, Mass.

² This oil was generously supplied by Mr. Melvin Hochberg of the Nopco Chemical Company, Harrison, New Jersey.

In the groups milked prepartum (2-A, 2-B) twice-daily milking was started 10 days prior to the calculated parturition date. Actually group 2-A was milked for an average of 9.6 ± 1.2 days prepartum, the two milkings immediately preceding parturition yielding 19.5 ± 3.8 lb. of milk. Similar values for group 2-B were 10.4 ± 1.4 days and 20.2 ± 3.1 lb. of milk. All cows were milked twice daily after parturition, in most cases beginning within 4 hours. The newborn

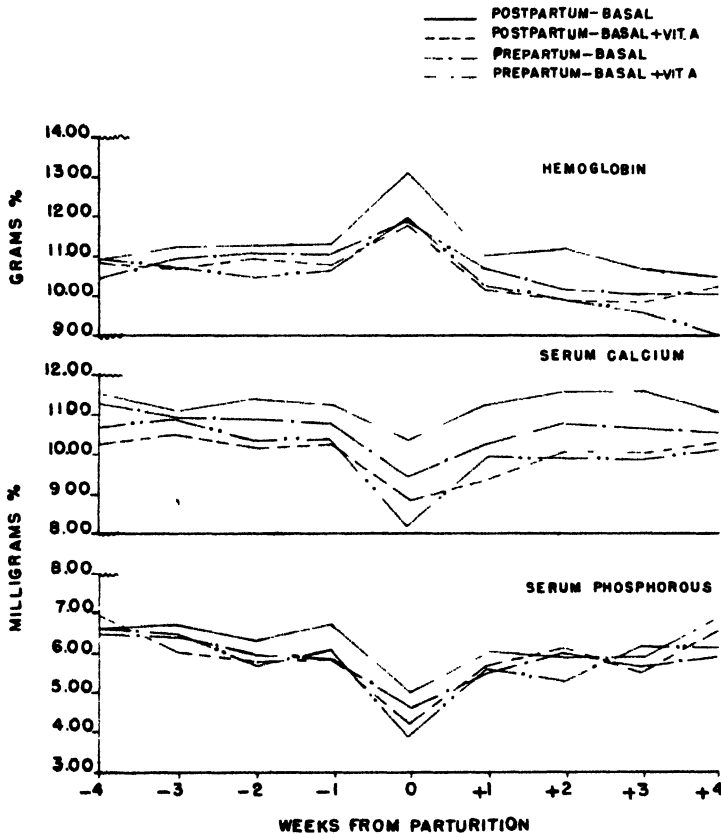


FIG. 1. The effect of prepartum milking on the hemoglobin and serum calcium and phosphorus levels at weekly intervals pre- and postpartum.

calves were removed immediately without nursing to a separate portion of the barn.

Samples and Observations. Venous blood samples were collected from all cows between 7 and 8 a.m. at weekly intervals for 4 weeks prepartum and for 4 weeks postpartum. An additional sample was obtained within 4 hours after parturition from each cow and followed by daily samples for 4 days in 26 of the cows. The sample volumes were 60 ml., of which one-half was citrated and one-half was allowed to clot. All samples were held at 4° C. until analyzed.

Total blood hemoglobin, carotene and vitamin A were determined immediately and the serum calcium and inorganic phosphorus usually within 72 hours.

The mammary and umbilical edema were graded independently on a scale from 0 to 10 by two of the authors (H.D.E. and R.E.J.) at the first milking postpartum. In addition the number of days for the edema to disappear was recorded. The milk fever and ketosis cases were diagnosed clinically by one of the authors (C.F.H.). These were confirmed by determinations of the serum calcium, blood sugar, and urine acetone and histological sectioning of liver biopsies for glycogen and fat. Placentas that were not completely expelled within 12 hours after parturition were recorded as retained. Two cows, one in group 1-B and one in

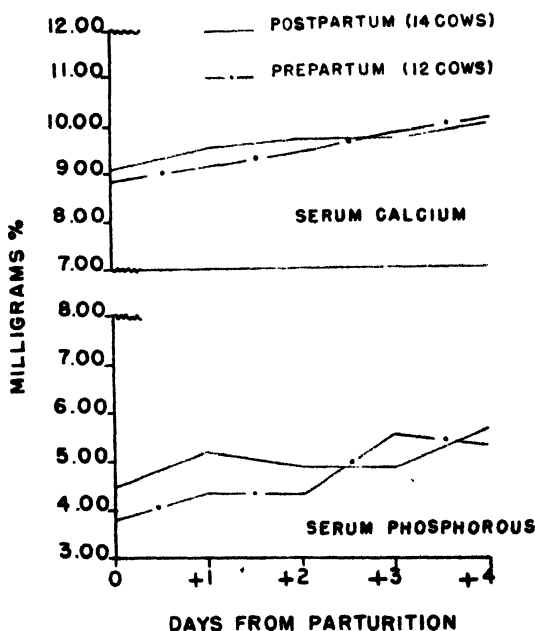


FIG. 2. The effect of prepartum milking on serum calcium and phosphorus levels at daily intervals postpartum.

group 2-B, were given 500 ml. of 20 per cent calcium gluconate intravenously for milk fever after taking the blood sample at parturition. A total of five cows was given intravenously varying amounts of 40 per cent dextrose for ketosis.

Analyses. Total hemoglobin was determined by the method of Evelyn and Malloy (5), total serum calcium by the method of Clark and Collip (2), total serum inorganic phosphorous by the method of Fiske and Subbarow (6) and plasma carotene and vitamin A by the method of Kimble (8). It is recognized that the method of Kimble underestimates the level of vitamin A in the presence of more than 300 γ carotene per 100 ml. plasma (1, 3, 13, 19). Standard statistical procedures (18), such as the analysis of variance and covariance, were used to test for difference between treatments.

RESULTS AND DISCUSSION

Data for all cows are given in figures 1 and 3 on the levels of hemoglobin, of serum calcium and phosphorous and of plasma carotene and vitamin A at parturition and at weekly intervals before and after. Values for serum calcium and phosphorous at parturition and for 4 days postpartum for 26 of the cows are included in figure 2. The scores for mammary and umbilical edema and some

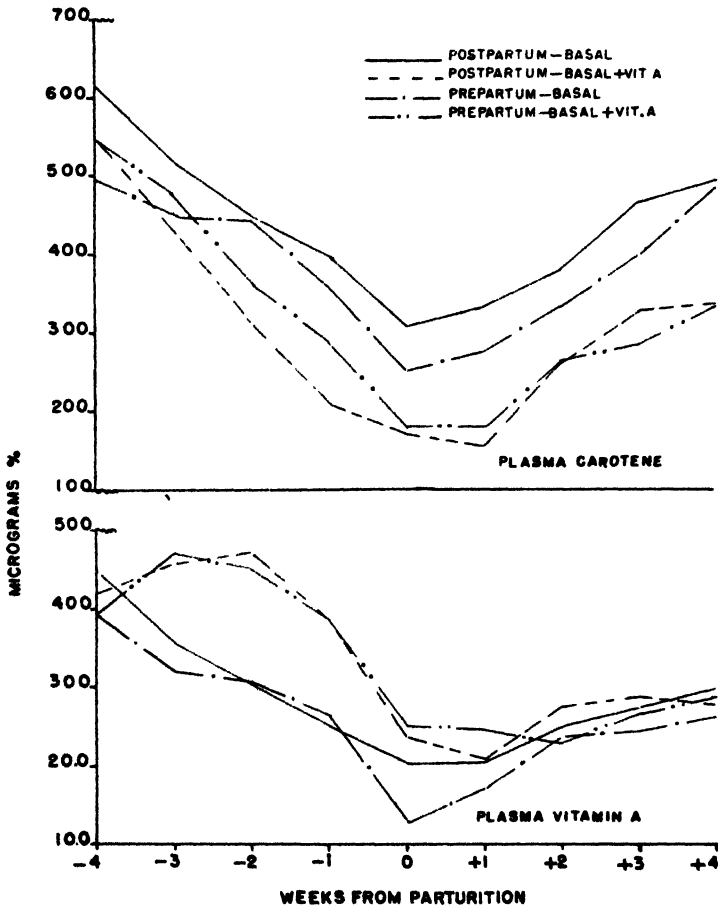


FIG. 3. The effect of prepartum milking on the plasma carotene and vitamin A levels at weekly intervals pre- and postpartum.

other physiological disturbances occurring at or near parturition are contained in table 1. In general prepartum milking did not affect significantly any of these criteria. The supplementary feeding of vitamin A prepartum influenced only the carotene and vitamin A levels in the blood plasma.

Hemoglobin. Total hemoglobin (fig. 1) was not influenced by either prepartum milking or the supplementary feeding of vitamin A. Since the greatest

change in hemoglobin, as well as in calcium and phosphorous, occurred at parturition the difference between the sum of the values observed 1 week before and 1 week after parturition and twice the value at parturition for each cow was used in an analysis of variance. This showed no real differences between treatments. Although not of primary concern in this experiment, the lower levels of hemoglobin occurring after parturition are of interest. With the exception of the group milked only postpartum and fed the basal ration, this decrease was statistically significant ($P > 0.05$).

TABLE 1

The effect of prepartum milking on edema and other physiological disturbances occurring at or near parturition

	Experimental Group			
	Postpartum		Prepartum	
	Basal (1-A)	Basal + Vit. A (1-B)	Basal (2-A)	Basal + Vit. A (2-B)
Number of animals	9	11	12	11
Mammary edema scores ^a	1.3 ± 0.3	1.9 ± 0.4	2.4 ± 0.4	2.6 ± 0.6
Days for edema to disappear ^b	5.4 ± 0.4	5.5 ± 0.5	10.7 ± 0.4	8.9 ± 0.5
Umbilical edema scores ^a	0.4 ± 0.2	0.5 ± 0.3	1.1 ± 0.3	1.0 ± 0.6
Days for edema to disappear ^b	1.2 ± 0.5	1.5 ± 0.5	3.7 ± 0.5	2.0 ± 0.6
Incidence of milk fever ^c	0	1	0	1
Incidence of ketosis ^c	1	3	0	1
Incidence of retained placenta ^c	3	3	4	4

^a Arithmetic mean \pm standard error of mean.

^b Geometric mean \pm standard error of mean.

^c Total number of cases.

The levels of hemoglobin observed in this study are in essential agreement with those reported recently (9, 14, 15). The marked rise in the hemoglobin at parturition may be due to a decrease in plasma volume associated with parturition. The decrease in the level of hemoglobin after calving may, in contrast, be due to an increase in plasma volume, since lactating cows have been reported (21) to have higher plasma (and blood) volumes per unit of liveweight than non-lactating cows.

Serum calcium and inorganic phosphorous. Neither serum calcium nor inorganic phosphorous (fig. 1 and 2) was affected by prepartum milking or the supplementary feeding of vitamin A. An analysis of the drop in serum calcium and in inorganic phosphorous at the time of parturition, similar to that described for hemoglobin, indicated no real differences between treatments. A more detailed study on 26 of the cows covering the 4 days after parturition gave similar results. This study and those of Smith *et al.* (16, 17, 12) indicate that the changes in serum calcium and inorganic phosphorous occurring at the time of parturition apparently are independent of the initiation of lactation.

Plasma carotene and vitamin A. The plasma levels of carotene and vitamin A were affected significantly by the supplementary prepartum feeding of vitamin A but were not influenced by prepartum milking. An analysis of covariance of the results on carotene used the third week values prepartum as initial levels

to adjust individual differences between cows and an average of the values for the 6 weeks thereafter as the response. This analysis indicated a highly significant ($P > 0.001$) depression in the carotene levels of the plasma of those groups fed supplementary vitamin A. The plasma values of vitamin A, excluding the 4th week prepartum, were higher ($P > 0.05$) in the groups fed supplementary vitamin A than in the groups fed the basal ration alone.

The trends in the levels of both carotene and vitamin A are similar to those reported previously (3, 20) for cows milked only postpartum. Keyes (7) observed a similar trend in plasma carotene for cows milked prepartum. The depression in plasma carotene when supplementary vitamin A is fed also has been demonstrated by others and the literature adequately reviewed by Esh *et al.* (3) and Wise *et al.* (22).

Mammary and umbilical edema. The degree of mammary and of umbilical edema (table 1) and the days for the edema to disappear were higher in those groups milked prepartum, although the difference was not statistically significant. The absence of any beneficial results of prepartum milking on edema does not agree with the report of Turner (20). In the animals reported herein, wide variability was observed between animals within an experimental group, indicating that edema associated with parturition was largely a matter of the individual animal rather than of treatment.

SUMMARY

The effect of prepartum milking for 10 days prior to the calculated parturition date on the total hemoglobin, serum calcium and inorganic phosphorous, plasma carotene and vitamin A, and mammary and umbilical edema has been studied in 43 cows. Secondly, the effect of feeding daily one million USP units of vitamin A for 30 days prior to the calculated parturition date was measured.

Prepartum milking had no significant effect on the changes occurring at parturition in the several blood constituents, nor did prepartum milking affect significantly the mammary and umbilical edema present at parturition. The prepartum feeding of supplementary vitamin A caused a significant decrease in plasma carotene and increase in plasma vitamin A.

ACKNOWLEDGMENT

The authors are most grateful to F. Warren and G. Farrington for the care of the experimental animals and to Misses R. J. Caverno and M. W. Dicks and to L. Nezvesky for technical assistance at various times during the course of the experiment. Further acknowledgement is due C. I. Bliss, Storrs Agricultural Experiment Station Biometrician, for considerable aid in the statistical analyses of the data and in the preparation of the paper and to Mrs. L. Griswold, Department of Poultry Husbandry, for the carotene analyses of the feedstuffs.

REFERENCES

- (1) BOYER, P. D., PHILLIPS, P. H., AND SMITH, J. K. The Separation of Carotene from Vitamin A. in Blood Plasma. *J. Biol. Chem.*, 152: 445-452. 1944.

- (2) CLARK, E. P., AND COLLIP, J. R. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with Suggested Modifications. *J. Biol. Chem.*, **63**: 461. 1925.
- (3) ESH, G. C., SUTTON, T. S., HIBBS, J. W., AND KRAUSE, W. E. The Effects of Soya-Phosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, **31**: 461-478. 1948.
- (4) ESPE, D. L. *Secretion of Milk*. 2nd. Ed. Pp. 142-143. Iowa State College Press, Ames. 1946.
- (5) EVELYN, K. A., AND MALLOY, H. T. Microdetermination of Oxyhemoglobin, Methemoglobin, and Sulfhemoglobin in a Single Sample of Blood. *J. Biol. Chem.*, **126**: 655-662. 1938.
- (6) FISKE, C. H., AND SUBBAROW, Y. The Colorimeter Determination of Phosphorus. *J. Biol. Chem.*, **66**: 375-400. 1925.
- (7) KEYES, E. A., REID, J. J., BECHDEL, S. I., BORLAND, A. A., BEAM, A. L., AND WILLIAMS, P. S. Pre-Partum Milking. (Abstract). *J. Dairy Sci.*, **27**: 638-639. 1944.
- (8) KIMBLE, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Plasma. *J. Lab. Clin. Med.*, **24**: 1055-1065. 1939.
- (9) MATRONE, G., PETERSON, W. J., BAXLEY, H. M., AND GRINNELS, C. D. Copper and Iron in the Blood Serum of Dairy Cows. *J. Dairy Sci.*, **30**: 121-126. 1947.
- (10) MOORE, L. A., AND ELY, R. Extraction of Carotene from Plant Material. A Rapid Quantitative Method. *Ind. Eng. Chem., Anal. Ed.*, **13**: 600-601. 1941.
- (11) NELSON, W. L., LOOSLI, J. K., LOPGREN, G., AND YAGER, N. Carotene Retention in Alfalfa Meal. Effect of Blanching, Packaging and Storage Temperature. *Ind. Eng. Chem.*, **40**: 2196-2198. 1948.
- (12) NIEDERMEIER, R. P., AND SMITH, V. R. A Study of Some Blood Constituents of Cows Not Milked Following Parturition. (Abstract). *J. Dairy Sci.*, **31**: 684-685. 1948.
- (13) PARRISH, D. B., WISE, G. H., AND HUGHES, J. H. Vitamin A and Carotenoids in the Blood Serum of Dairy Cattle. Chemical Methods for Determination. *Anal. Chem.*, **20**: 230-233. 1948.
- (14) REID, J. T., WARD, G. M., AND SALINHURY, R. L. Mineral Metabolism Studies in Dairy Cattle. IV. Effects of Mineral Supplementation of the Prepartal Diet Upon Composition of the Blood of Cows and Their Calves At Parturition. *J. Nutrition*, **36**: 75-90. 1948.
- (15) RUSOFF, L. L., AND PIERCY, L. L. Blood Studies of Louisiana Dairy Cows. II. Calcium, Inorganic Phosphorus, Hemoglobin, Value, Erythrocyte Count, Leucocyte Count, and Differential Leucocyte Percentages. *J. Dairy Sci.*, **29**: 831-838. 1946.
- (16) SMITH, V. R., AND BLOSSER, T. H. Parturient Paresis. I. The Incidence of Parturient Paresis and Changes in the Total Blood Serum Calcium at Parturition in Prepartum Milked Cows. *J. Dairy Sci.*, **30**: 861-866. 1947.
- (17) SMITH, V. R., NIEDERMEIER, R. P., AND HANSEN, R. G. Parturient Paresis. II. The Effect of Partial Versus Complete Milking Upon The Total Blood Serum Calcium of Dairy Cows at Parturition. *J. Dairy Sci.*, **31**: 173-177. 1948.
- (18) SNEDCOR, G. W. *Statistical Methods*. 4th Ed. Iowa State College Press, Ames, Iowa. 1946.
- (19) SUTTON, T. S., KAESER, H. E., AND SOLDNER, P. A. Changes in Level of Vitamin A and Carotene in the Blood Plasma of Dairy Cows Associated with Parturition and Beginning Lactation. *J. Dairy Sci.*, **28**: 933-939. 1945.
- (20) TURNER, C. W. The Development of the Mammary Gland as Indicated by the Initiation and Increase in the Yield of Secretion. *Mo. Agr. Expt. Sta. Research Bull.* 156. 1931.
- (21) TURNER, C. W., AND HERMAN, H. A. A Determination of the Blood and Plasma Volume of Dairy Cattle. *Mo. Agr. Expt. Sta. Research Bull.* 159. 1931.
- (22) WISE, G. H., ATKESON, F. W., CALDWELL, M. J., PARRISH, D. B., AND HUGHES, J. S. Effect of High Vitamin A Intakes on Milk and Fat Yields and On Vitamin A Contents in Milk, Blood, and Livers of Dairy Cows. *J. Dairy Sci.*, **30**: 279-291. 1947.

NICKEL IN COWS' MILK.¹

J. G. ARCHIBALD

Massachusetts Agricultural Experiment Station, Amherst

This paper is the fifth in a series on the mineral elements of cows' milk (2, 3, 4, 5). Previous investigators (1, 7, 8, 9, 14, 15) are almost unanimous in agreement that nickel, unlike cobalt and certain other trace elements, is not present in milk. Only one group (1) reports its presence (in evaporated milk); the others, with one exception (8), do not make any positive statement about its absence—it simply is not listed among those elements revealed by spectrographic analysis of milk ash. In the exceptional case referred to above (8), the authors state that "no evidence has been obtained of any of the following elements in pure milk". There follows a list of 34 elements in which both cobalt and nickel appear, together with the comment that "the complete absence of cobalt is somewhat unexpected, for it is an element with active biological properties". Cobalt has since been found in cows' milk by several investigators (4, 6, 12, 13), and since cobalt and nickel are so closely related in the periodic system, it has been thought worth while to include nickel in the series of elements under investigation here. The objective has been two-fold: (a) to determine whether nickel is naturally present in milk, and (b) to find out whether, if present, the amount of it can be increased by feeding a nickel compound to cows.

EXPERIMENTAL

The procedure was similar to that described in an earlier paper (2). The work was carried on during two winter feeding seasons, eight cows being used in 1948 as in earlier work, and six in 1949. The four breeds represented in each sub-group of cows in 1948 were the Ayrshire, Guernsey, Holstein and milking Shorthorn. In 1949 the last three mentioned were used. Each breed pair was quite closely matched with respect to age and stage of lactation. The supplement fed was nickel (ous) chloride ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) in approximate daily amount of 500 mg., which is the equivalent of 145 mg. of elemental nickel. One group received the supplement during December and January in 1947-48; the other group received it during February and March. In 1949 the feeding trial was started a month later, so that one group received the supplement during January and February and the other during March and April. The reason for repeating the trials will appear in a moment.

Composite 2-day milk samples of 2 l. each were taken from each cow once a month. Nickel was determined in triplicate on 500-ml. portions of each sample by the method of Alexander *et al.* (1). This method, when used in conjunction with a quartz spectrophotometer, working with standard solutions of nickel chloride and known amounts of added nickel, gave good results. Results the

Received for publication June 16, 1949.

¹ Contribution No. 727 of the Massachusetts Agricultural Experiment Station.

first year were obtained with the aid of a grating type of spectrophotometer and were not uniform. Admittedly the grating type of instrument is not as delicate as the quartz type, and it was therefore deemed advisable to repeat the work a second season using an instrument² that would more adequately resolve the very minute differences involved. In addition, as a preliminary to work of the second season, each step in the analytical procedure was subjected to rigorous inquiry in order to refine technique wherever possible.

RESULTS

The values obtained are summarized in the accompanying tables, table 1 containing the results for 1948, and table 2 those for 1949. In the 1948 results, al-

TABLE 1

*Effect on nickel content of the milk of feeding cows nickel chloride (season of 1947-48)
(γ of nickel/l. of milk)*

Month	Cows on control ration					Cows receiving supplemental nickel				
	1st half of the season									
	A232	G639	H447	S74	Av.	A205	G629	H444	S62	Av.
December	12.8	20.2	10.6	1.6	11.3	8.8	22.6	20.0	21.1	18.1
January	10.0	5.2	1.2	2.8	4.8	3.2	2.8	0	25.0	7.8
Av.—1st half	11.4	12.7	5.9	2.2	8.1	6.0	12.7	10.0	23.1	13.0
	2nd half of the season									
	A205	G629	H444	S62	Av.	A232	G639	H447	S74	Av.
February	1.2	0.5	10.4	0	3.0	6.4	0.8	0.6	6.0	3.5
March	1.4	1.4	0.8	0.3	1.0	1.8	1.8	20.4	1.6	6.4
Av. 2nd half	1.3	1.0	5.6	0.2	2.0	4.1	1.3	10.5	3.8	4.0
Av.—entire season	6.4	6.9	5.8	1.2'	5.1	5.1	7.0	10.3	13.5	9.0

* The initial letter prefixed to each cow's number indicates the breed.

though the trend was toward a higher concentration of nickel in the milk when the supplement was fed (9.0 γ /l. on the average, in contrast to 5.1 γ), as already noted the individual values were entirely lacking in uniformity (5 of the 16 possible comparisons were in the reverse direction) and because of the wide variations the difference in the general averages (3.9 γ /l.) was not significant statistically. It therefore was decided to repeat the work, and it will be noted from table 2 that the results were even more inconclusive than in 1948. The cows showed a slightly higher average amount of nickel in their milk when not receiving the supplement than they did when they received it; there was even greater variability in the individual results; and the small average difference was statistically not sig-

² The instrument used in this later work was a Model DU Beckman spectrophotometer. Measurements were made at a wave length of 385 μ , using Corning filter #9863 (red purple Corax A).

nificant. The values for the 1949 season are in general of a much lower magnitude than those for 1948. This is attributed to refinements of technique and method and to the use of a more precise spectrophotometer.

Obviously these inconclusive and conflicting results in 2 successive years mean one of two things; either cows do not secrete supplemental nickel uniformly into their milk as they do cobalt (4), or else natural milk does not contain nickel at all, in which case the widely varying amounts found represent varying amounts of nickel dissolved from the milking machine. It was decided to either eliminate or confirm this latter possibility.

TABLE 2

*Effect on nickel content of the milk of feeding cows nickel chloride. (season of 1949)
(γ of nickel/l. of milk)*

Month	Cows on control ration				Cows receiving supplemental nickel			
	1st half of the trial							
	G692	H567	S74	Av.	G686	H444	S68	Av.
January	0.48	1.36	0.48	0.77	0.20	1.12	0.40	0.57
February	0.52	1.04	1.76	1.11	0.96	0.56	0.48	0.67
Av.—1st half	0.50	1.20	1.12	0.94	0.58	0.84	0.44	0.62
	2nd half of the trial							
	G686	H444	S68	Av.	G692	H567	S74	Av.
March	0.24	2.24	1.12	1.20	1.40	2.20	0.88	1.49
April	0.32	0.72	1.54	0.86	0.24	0.72	0.66	0.54
Av.—2nd half	0.28	1.48	1.33	1.03	0.82	1.46	0.77	1.02
Av.—entire season	0.39	1.34	1.23	0.99	0.70	1.15	0.61	0.82

At the conclusion of the feeding trial in April, 1949, composite samples for two milkings from each sub-group of cows were obtained without any contact with metal by milking by hand directly into 2-l. glass jars after the fore-milk had been first drawn off and discarded. These samples when carried through the usual analytical procedure were found to contain not even a slight trace of nickel.

The conclusion from this final phase of the investigation hardly needs stating, but it raises an interesting question. Here are two elements—cobalt and nickel—side by side in the periodic system; their atomic weights and atomic numbers are respectively: 58.9 and 58.7, 27 and 28. The only known difference in structure is a difference of one electron in the "M" orbit; cobalt has 15 electrons in this orbit, while nickel has 16. In the past few years it has been shown by numerous investigators that cobalt has a remarkable biological significance and within a year it has been proven to be a constituent of vitamin B₁₂ (11). Nickel on the other hand is not known to be essential to biological systems, and here is further evidence of the same sort; viz. that either it is not absorbed into the blood stream or else the mammary gland excludes it while permitting cobalt to pass into

the milk. A similar situation with respect to iron and manganese was noted and commented on in an earlier paper of this series (5).

SUMMARY

Nickelous chloride was fed as a supplement (500 mg. daily) to the rations of six cows for a period of 2 months by the double reversal method and the milk was analyzed for nickel. Although varying amounts of nickel were found in the numerous milk samples taken, it was shown that when the milk was kept from contact with metal by milking directly into glass jars, nickel was not present.

Therefore it was concluded that nickel is not a constituent of natural milk and that the varying amounts found in the course of the investigation came from the milking machine.

The difference in this respect between cobalt and nickel, two closely related elements, is discussed briefly.

REFERENCES

- (1) ALEXANDER, O. R., GODAR, E. M., AND LINDE, N. J. Spectrophotometric Determination of Traces of Nickel. *Ind. Eng. Chem. (Anal. Ed.)* **18**: 206-208. 1946.
- (2) ARCHIBALD, J. G. Manganese in Cows' Milk. *Milk Plant Monthly*, **30**, (9): 36. 1941.
- (3) ARCHIBALD, J. G. Zinc in Cows' Milk. *J. Dairy Sci.*, **27**: 257-261. 1944.
- (4) ARCHIBALD, J. G. Cobalt in Cows' Milk. *J. Dairy Sci.*, **30**: 293-299. 1947.
- (5) ARCHIBALD, J. G., AND LINDQUIST, H. G. Manganese in Cows' Milk. *J. Dairy Sci.*, **26**: 325-330. 1943.
- (6) ASKEW, H. O. Mineral Content of Pastures. Investigations at the Cawthron Institute. *New Zeal. Dep. Sci. Ind. Res., Ann. Rpt.*, **16**: 13-15. 1942.
- (7) BLUMBERG, H., AND BASK, O. S. The Spectrographic Analysis of Milk Ashes. *J. Nutr.*, **6**: 285-288. 1933.
- (8) DINGLE, H., AND SHELDON, J. H. A Spectrographic Examination of the Mineral Content of Human and Other Milk. *Biochem. J.*, **32**: 1078-1086. 1938.
- (9) DREA, W. F. Spectrum Analysis of Milk Ashes. *J. Nutr.*, **8**: 229-234. 1934; also, **16**: 325-331. 1938.
- (10) ELLIS, G. H., AND THOMPSON, J. F. Determination of Cobalt in Biological Materials with Nitrosocresol. *Ind. Eng. Chem. (Anal. Ed.)* **17**: 254-257. 1945.
- (11) RICKES, E. L., BRINK, N. G., KONIUSZY, F. R., WOOD, T. R., AND FOLKERN, K. Vitamin B₁₂, a Cobalt Complex. *Science*, **108**: 134. 1948.
- (12) SYLVESTER, N. D., AND LAMPITT, L. H. The Determination of Cobalt in Foods. *Soc. Chem. Ind. J., Trans. and Commun.*, **59**: 57-60. 1940.
- (13) UNDERWOOD, E. J., AND ELVEHJEM, C. A. Is Cobalt of Any Significance in the Treatment of Milk Anemia with Iron and Copper? *J. Biol. Chem.*, **124**: 419-424. 1938.
- (14) WRIGHT, N. C., AND PAPISH, J. The Inorganic Constituents of Milk. *Science*, **69**: 78. 1929.
- (15) ZBINDEN, C. The Infinitely Small Quantities of Certain Elements in Milk and Their Detection by the Spectrographic Method. *Lait*, **11**: 114. 1931.

THE METABOLISM OF THE LACTOGENIC¹ HORMONE¹

T. Y. LIU AND C. W. TURNER

Department of Dairy Husbandry, University of Missouri, Columbia

The lactogenic hormone secreted by the anterior pituitary gland is essential for the secretion of milk. It has been suggested that the initial increase in the secretion of this hormone at about the time of parturition is due to the action of estrogen upon the pituitary gland, and that the maintenance of lactogen secretion is largely due to the regular stimulation of the teats at the time of milking (16).

The mode of action of the lactogenic hormone upon the epithelial cells of the mammary gland is not understood, but it is presumed to act upon the enzyme systems of the cells which cause the milk precursors coming from the blood to be transformed into the several constituents of milk (21).

Since the intensity of lactogenic hormone secretion by the pituitary undoubtedly is one of the important factors governing the inheritance of the capacity for large milk and fat production, it is important that methods be developed to measure the secretion rate of this hormone. In experimental animals where they may be sacrificed, the determination of the amount of the lactogenic hormone present in the pituitary under varying physiological conditions has been studied extensively. In these studies, there has been observed a high degree of relation between the amount of lactogenic hormone present in the pituitary and the rate of milk secretion.

In the larger domestic animals it is impossible to sacrifice the animals, and other methods must be developed to measure lactogenic hormone secretion rate. Ehrhardt and Voller (3) assayed the blood and urine for lactogen during the menstrual cycle. They claimed that parallel peaks in both blood and urine were observed at the beginning of menstruation and at the time of ovulation. It has been shown, also, that the lactogenic hormone is present in the blood of dairy cattle, goats and rabbits (20), and in the serum of mares during pregnancy and lactation (9). Meites and Turner (14) assayed the whole, untreated blood of rabbits before and after the injection of estrone. There was a definite increase in the lactogen content of the blood associated with an increase in the pituitary.

During recent years, the problem of hormone metabolism has been under intensive investigation. There are many aspects of the problem including the question of the use of the hormone by the cells which are activated, the inactivation of the hormone in other parts of the body but especially in the liver, and the paths of elimination.

Lyons and Page (13) were the first to report the presence of lactogenic hormone in postpartum human urine. They claimed that at least as much of this hormone is excreted daily as is present in the bovine pituitary. Lyons (12) also showed that lactogen was present in the urine of four new-born male and

Received for publication June 16, 1949.

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 1156.

female babies, who were secreting witches milk. Tesauro (19) found that the injection of untreated or extracted postpartum urine from women gave a definite increase in the crop-gland weight of pigeons. Hoffmann (6) reported no hormones could be detected in women's urine before parturition in 10 of 11 cases. Following parturition no hormone was detected for the first 2 days, but beginning on the third day, with the inflow of milk, lactogen could be demonstrated in the urine. Hoffmann (7) further found that in seven of eight cases of deficient lactation, the excretion of lactogen was considerably less than in the urine of women with ample lactation.

Langecker and Schenk (8) detected lactogen in the urine of women during lactation, and also at the end of pregnancy. One and one-half liters of urinary extract was found to be as effective as 1.8 g. of fresh cattle pituitary.

Liard (11) reported that there was sufficient lactogen in 50 to 150 ml. samples of urine from lactating women to produce milk secretion in guinea pigs primed with large doses of estrogen.

The lactogen content of the urine of ten lactating women during the first 2 weeks postpartum was determined by Meites and Turner (15). They observed a daily range in hormone excretion of from 4.05 to 12.50 I. U. From 0.8 to 3.0 ml. of urine was required for a micro-unit response. In three cases of definite or suspected hypogalactia less lactogenic hormone was found than in mothers with adequate lactation. The three best lactators excreted the highest average amount of hormone.

Coppedge and Segaloff (2) found that the lactogenic hormone content of urine collected during 24-hour periods from normal women ranged from 20 to 100 I. U. and from normal males ranged from 50 to 100 I. U.

Stimulated by the discovery of rather large amounts of male hormone in dried cow manure [for references see (23)], it seemed of interest to determine whether or not other hormones might be excreted into the digestive tract. Since the lactogenic hormone is a protein hormone, it might be thought unlikely that it would be present in the feces for two reasons. First, if the hormone passed into the digestive tract it might be partially or totally digested and rendered inactive. Second, that protein would pass unaltered through the digestive mucosa in the reverse direction. That the lactogenic hormone passes into the urine in a biologically active form is proof of the passage through the kidney epithelium.

The object of the present paper is to describe a method which successfully extracted lactogen from dried cow manure, and to report upon the content of the hormone in the feces of cows in varying stages of lactation. Its presence in the feces of bulls also was observed.

MATERIALS AND METHODS

The fresh cow manure free from urine, straw and extraneous material was collected and dried by spreading it in a thin layer in an electrical drying oven at a temperature of 45° C. for about 48 hours. The sample then was ground in a mill to the consistency of alfalfa meal and then was ready for extraction.

Bergman and Turner (1) made a study of four methods frequently employed

for the extraction of lactogen. They found aqueous alkaline ethyl alcohol was satisfactory for the extraction of lactogen, and also for the other pituitary hormones. It was decided to use ethyl alcohol as the solvent for the extraction of the hormones which might be present in the cow manure.

Initial attempts were made to obtain the protein hormones qualitatively from the manure of the lactating cow by successive extraction with 90, 80, 70, 60, and 50 per cent ethyl alcohol. These extracts were divided into several portions for further study. The concentration of alcohol was increased in most cases and an attempt was made to precipitate any hormones which might be present by adjusting the pH to their individual isoelectric point.

A positive qualitative response for the lactogenic hormone was obtained. Negative assays for the adrenocorticotrophic, thyrotrophic and gonadotrophic hormones were obtained. It was decided to extract the manure for the lactogenic hormone without regard to the other hormones and to compare the lactogenic hormone excretion by way of the digestive tract from bulls and high and low milk producing cows.

In the beginning a few attempts were made to precipitate the active material from the alcohol extract suspension by tannic acid, benzoic acid as used for precipitating the protein hormones from urine, and by increasing the concentration of the alcohol solution. It was found that increasing the alcohol concentration was the most effective method. It was also found that extraction with 40 per cent ethyl alcohol was most effective. The steps are as follows:

1. A 1 kg. sample of dried cow manure which was collected individually was mixed thoroughly with 10 l. of 40 per cent ethyl alcohol.

2. The suspension was brought to pH 10 (Beckman glass electrode) by the addition of 1 N NaOH solution, and was kept at room temperature for 4 hours with frequent stirring.

3. The residue was removed by the aid of a centrifuge fitted with a perforated disc and a draining chamber. One-fifth of the original quantity of alcohol can be obtained by this method.

4. The fine particles in the suspension were removed by centrifuging in large centrifuge bottles.

5. The combined clear supernatant fluid was then filtered through a no. 1 filter paper on a Buchner funnel with suction.

6. A volume of 90 per cent alcohol was added so as to increase the alcohol concentration to 65 per cent and brought immediately to pH 6.5 by the addition of 1 N HCl solution. It then was kept at a temperature of -1 to -5° C. for 12 hours, at the end of which period the precipitate had settled down to the bottom of the container.

7. The supernatant solution was sucked out by the aid of vacuum. The precipitate together with a small quantity of fluid was poured into large centrifuge bottles which were kept at -1 to -5° C.

8. The solution was centrifuged and the precipitate discarded. A one-half volume of 95 per cent ethyl alcohol was added to the combined supernatant solution so as to increase the alcohol concentration to 75 per cent. The mixture was

brought to pH 5.7 by the addition of 1 *N* HCl and kept at a temperature of -1 to -5° C. for 12 hours, at the end of which period the active material had settled down to the bottom of the container.

9. The supernatant solution was sucked out and a small amount of fluid was separated from the precipitate by centrifuging under cold conditions and was discarded.

10. The precipitate was dissolved in distilled water by adjusting the pH to 9 and was dialyzed against distilled water for 24 hours. For dialyzing, a cellulose casing was used. It was soaked in distilled water for 5 to 10 minutes, tied at one end, filled with solution, and then the other tied, and placed in a large beaker containing distilled water which was changed several times. The beaker was kept under cold conditions. By this step the active material loses much of its toxic and inflammatory properties.

11. After dialyzing, 5 volumes of 95 per cent alcohol were added to the dialyzed solution which was brought to pH 5.7 by the addition of 1 *N* HCl and kept at -1 to -5° C. for a few hours.

12. The precipitate was collected by centrifugation and was washed four times with 95 per cent alcohol and three times with ether.

13. After the last washing, the precipitate was easily dried by holding the centrifuge bottle in front of an electrical fan. The grayish dry material was ground to pass through an 80 mesh sieve and was then ready for biological assay.

To determine the amount of lactogenic hormone present, the very sensitive "micro" assay was used (15). It is based upon the proliferation of the crop gland of pigeons after intradermal injection over the crop glands. The dried extracts which were screened through an 80-mesh sieve were dissolved in distilled water and adjusted to pH 9 with NaOH solution. After the extract was completely dissolved the solution was brought to the isoelectric point with HCl so as to precipitate the protein and to slow down the absorption rate from the site of injection. The volume of fluid injected daily amounted to 0.1 ml. In all quantitative assays, preliminary tests were made at three dosage levels in a few pigeons in order to approximate the unit. The "micro" unit is defined in our laboratory as that amount of hormone which, when injected intradermally over the crop gland of 20 pigeons, will elicit a minimum but definite response in 50 ± 10 per cent of the pigeons. This unit is equivalent to 1/160 I. U. of lactogenic hormone.

RESULT OF QUANTITATIVE ASSAY OF LACTOGENIC HORMONE

Quantitative assays for lactogen were made on the extracts of cow manure from individual cows. It is shown (Table 1) that in general the cows producing considerable milk excreted more lactogenic hormone through their digestive tract than the low producing cows except in the case of H 139, which was a relatively good milk producer, yet excreted less lactogenic hormone than the low producer H 95. The lactogenic hormone content of 1 kg. dry manure of high-producing cows ranged from 47.9 to 200 "micro" units (0.29 to 1.25 I. U.),

TABLE 1

Correlation between milk record and the lactogenic hormone content of bovine manure

Records of Dairy Cattle					Yield of Extract	Lactogenic Hormone Content of cow manure		
Animal no.	Sex	Monthly milk production when sample was collected	From freshening to the time of sample collection		Av. total yield/kg. of sample	Mg./micro unit	Av. total unit/kg. of sample	
			Total milk production				Micro unit	International unit
		(lb.)	(lb.)	(d.)	(mg.)			
J977	F.	645.0	645.0	27	3200.0	20	160.0	1.00
H139	F.	1064.6	2599.6	68	1150.0	24	47.9	0.29
H46	F.	460.2	7362.3	247	2400.0	24	100.0	0.62
H63	F.	1468.0	5456.9	104	2020.0	24	84.1	0.52
H84	F.	1030.0	5964.0	130	1300.0	16	81.2	0.50
H81	F.	1427.0	1625.0	35	3600.0	18	200.0	1.25
J987	F.	494.6	4095.0	243	1180.0	32	36.8	0.23
J994	F.	440.9	2600.3	186	1208.0	28	43.1	0.26
H95	F.	383.7	6729.0	265	1156.0	24	48.1	0.30
H	M.				1022.0	36	28.0	0.17
H	M.				1000.0	32	31.0	0.19

while the low producers ranged from 36.8 to 48.7 "micro" units (0.23 to 0.3 I. U.). The two bulls excreted nearly as much as the low milk-producing dairy cows, *i.e.* 31.2 and 28.3 micro units (0.19 and 0.17 I. U.), respectively.

DISCUSSION

The lactogenic hormone content of the pituitary remains low during most of pregnancy in experimental animals, but there is an abrupt increase shortly before or after parturition. Also the urinary lactogen content of lactating women increases after parturition and usually is related to the ability to secrete milk. In most experimental animals except guinea pigs, the pituitary lactogenic hormone content of females is higher than the males.

Although no experimental data are available on the urinary lactogenic hormone content of lactating dairy cattle, this investigation of the lactogenic hormone content of dried manure has shown that there may be a relationship between the milk producing capacity and the lactogenic hormone excretion from the digestive tract.

The discovery of lactogenic hormone in dairy cow manure raises the question of how and where this hormone is secreted into the digestive tract. The routes of excretion of metabolic by-products and the hormones in their active or inactive forms from the living body are:

(a). The urinary system through which the water, minerals, protein metabolic products, pigments, toxic substances, protein hormones such as gonadotrophin, adrenocorticotrophin, thyrotrophin, and steroid hormones such as estrogen, androgen and inactivated progesterone known as pregnandiol are excreted.

(b). The respiratory system through which water and carbon dioxide are eliminated.

(c). The sweat glands by which the sweat is secreted. The composition of sweat is nearly the same as the urine, but to what extent the hormones are excreted by the sweat glands is still a question.

(d). The digestive tract and its accessory organs such as the liver. The liver secretes bile which is poured into the digestive tract. In the bile the steroid hormone estrogen (17) has been found. Considerable amounts of estrogen (10) and androgen (23) have been found in cow manure.

Of great interest is the fact that the epithelial cells of the intestine have both absorptive and secretory functions. Nutrients are absorbed by these cells into the blood, while digestive enzymes, mucin and several hormones such as secretin, cholecystokinin, enterogastrone, villikin, etc., are formed by these cells and poured into the lumen of the intestine.

The lactogenic hormone is protein in nature and is manufactured by the anterior pituitary from where it is carried by the blood stream to the mammary gland. As it has been found in colostrum (5) of cows lactating normally, it is known that this hormone can be picked up by the mammary gland from the blood and excreted together with milk, so it is possible for the epithelial cells of the intestine to function in a similar manner. There might be another possibility for lactogenic hormone to reach the intestinal lumen if it were present in the bile.

Due to the fact that lactogenic hormone is inactivated by both pepsin and trypsin, it would be reasonable to suggest that in case it is excreted by the epithelial cells of the intestine, only that part which is excreted in the lower portion of the intestinal tract would be present in the feces in an active form unless it were protected from the digestive enzymes in some way.

It would be of interest to estimate the daily excretion of lactogenic hormone through the digestive tract. A dairy cow weighing 1000 lb. has been reported to excrete daily 59 lb. (4) of fresh manure. As the dry matter content of fresh manure is about 15 per cent (22), this would equal about 4.02 kg. dry matter per day. The average lactogenic hormone content per kg. of dry matter as indicated by these data is 0.54 I. U., so the daily lactogenic hormone excretion would be 2.17 I. U. The average lactogenic hormone content of the pituitary of the dairy cow has been estimated to be about 56.28 I. U. (18). Thus, 3.8 per cent of the lactogen contained in an average bovine pituitary is excreted in an active form through the digestive tract.

SUMMARY

1. Cow manure free from urine and extraneous materials was collected and dried in an electrical drying oven at a temperature of about 45° C. for about 48 hours.

2. Forty per cent aqueous alkaline ethyl alcohol extracts of individual samples of lactating cow and bull manure were precipitated by increasing the alcohol concentration to 75 per cent at pH 5.7 under cold conditions.

3. The average yield of lactogenic hormone from one kilogram dry manure ranged from 0.29 to 1.25 international units for the high milk producers, from

0.23 to 0.3 international units for the low milk producers, and from 0.17 to 0.19 international units for the bulls.

4. It was concluded that there might be a relationship between the amount of lactogenic hormone excretion into the digestive tract and the milk producing ability.

5. Possibly lactogenic hormone is secreted into the digestive tract, but only that part which is excreted in the lower portion of the intestine is excreted together with the feces in an active form.

REFERENCES

- (1) BERGMAN, A. J., AND TURNER, C. W. Comparison of Methods of Extraction of the Lactogenic Hormone. *J. Biol. Chem.*, **118**: 247. 1937.
- (2) COPPEDGE R. F., AND SEGALOFF, A. A Method for Assay of Prolactin in Human Urine. The Association for the Study of Internal Secretion, Thirty-first Meeting, 11. 1949.
- (3) EHRHARDT, K., AND VALIER, H. F. Untersuchungen über das Laktationehormon des Hypophysenvorderlappens. *Endokrinologie* **22**: 19. 1939.
- (4) FULLER, J. M. Some Physical and Physiological Activity of Dairy Cows. *New Hampshire Tech. Bul.* **35**. 1928.
- (5) GESCHICKTER, C., AND LEWIS, D. Lactogenic Substance in the Human Breast. *Arch. Surg.*, **32**: 598. 1936.
- (6) HOFFMANN, F. Ueber die Entstehung der Laktation. *Zentr. für Gynäkol.*, **60**: 2882. 1936.
- (7) HOFFMANN, F. Über die hypophysäre Hypogalaktie. *Zentr. Gynäkol.*, **61**: 35. 1937.
- (8) LANGECKER, H., AND SCHENK, P. Über die Ausscheidung des die Milchsekretion fördernden Hypophysenvorderlappenteils im Frauenbarn. *Med. Klin.*, **32**: 1104. 1936.
- (9) LEBLOND, C. P. Présence de traces de prolactine dans le serum de la jument durant la gravidité et la lactation. *Compt. rend. soc. biol.*, **124**: 1062. 1937.
- (10) LEVIN, L. The Fecal Excretion of Estrogens by Pregnant Cows. *J. Biol. Chem.*, **157**: 407. 1945.
- (11) LIARD, J. Sur la présence d'une substance lactogene dans les urines de la femme en lactation. *Compt. rend. soc. biol.*, **126**: 512. 1937.
- (12) LYONS, W. R. The Hormone Basis of "Witches Milk." *Proc. Soc. Exptl. Biol. Med.*, **37**: 207. 1937.
- (13) LYONS, W. R., AND PAGE, E. Detection of Mamotropin in the Urine of Lactating Women. *Proc. Soc. Exptl. Biol. Med.*, **32**: 1049. 1935.
- (14) MEITES, J., AND TURNER, C. W. Effect of Estrone on Lactogen Content in Pituitary and Blood of Male Rabbits. *Proc. Soc. Exptl. Biol. Med.*, **49**: 190.
- (15) MEITES, J., AND TURNER, C. W. Extraction and Assay of Lactogenic Hormone in Postpartum urine. *J. Clin. Endocrinology*, **1**: 918. 1941.
- (16) MEITES, J., AND TURNER, C. W. Studies Concerning the Induction and Maintenance of Lactation. I. The Mechanism Controlling the Initiation of Lactation at Parturition. *Mo. Agr. Exptl. Sta. Res. Bull.* **415**; II. The Normal Maintenance and Experimental Inhibition and Augmentation of Lactation. *Mo. Agr. Expt. Sta. Res. Bull.* **416**. 1948.
- (17) PEARLMAN, W. H., RAKOFF, A. E., CANTAROW, A., AND PASCHKIS, K. E. Isolation of Estrone from the Bile of Pregnant Cows. *J. Biol. Chem.*, **170**: 173-179. 1947.
- (18) REECE, R. P., AND TURNER, C. W. The Lactogenic and Thyrotrophic Hormone Content of the Anterior Lobe of the Pituitary Gland. *Mo. Agr. Expt. Sta. Res. Bull. No.* **266**. 1937.
- (19) TESAURIO, G. Contributo allo studio dell ormone gallatogeno (Ricerca nel sangue e nelle urine di donna in allattamento). *La Pediatrica*, **44**: 665. 1936.

- (20) TURNER, C. W. Galactin in the Blood of Cattle, Goats and Rabbits. Mo. Agr. Expt. Sta. Bull. No. 358, p. 52. 1934.
- (21) TURNER, C. W. Physiology of milk secretion. II. The Precursors of Milk. Chapt. II. *Bovine Mastitis*. Ed. by Little and Plastringe. McGraw-Hill Book Co., N. Y. 1946.
- (22) TURNER, C. W. Effect of Temperature and Drying on Activation of Male Hormone of Cow Manure. J. Dairy Sci. (In Press). 1949.
- (23) TURNER, C. W. Some Factors Influencing the Male Hormone Content of Cow Manure. J. Dairy Sci., 31: 1032. 1949.

VENOUS CATHETERIZATION OF DAIRY COWS

N. P. RALSTON, MAX KLEIBER AND A. H. SMITH¹
Division of Animal Husbandry, University of California, Davis

In studying the phosphorous metabolism of dairy cows by the use of radioactive tracers (16), it was necessary to develop a suitable injection and bleeding technique which would fulfill the following requirements: (a) It must permit complete injection of the radioactive material for hazard control; (b) it must provide for accurate and rapid injection of the material, followed immediately with the withdrawal of blood samples; (c) it must allow for the bleeding at frequent intervals over long periods (4 to 5 weeks); and (d) it must minimize the disturbance and apprehension of the cow, conditions which have been shown to affect the blood phosphorous values.

REVIEW OF LITERATURE

Forssman (9) in 1929 catheterized the right auricle on himself, after exposure of a vein of the arm by a surgeon. From 1930 to 1939 this technique (4) of catheterizing the right heart was widely used in Europe for injecting contrast substances in order to visualize the right chambers of the heart and the pulmonary vascular tree. Cournand and Ranges (2) modified the Forssman technique. They used a specially made gauge (no. 10) Lindeman type of needle; a three way stopcock with a Leur lock; a tightly fitted adapter; and a No. 8 F flexible, X-ray opaque, varnished, silk catheter with two holes, one at the rounded tip and another about 1 cm. from the tip. A saline reservoir with rubber tubing and a clamp for controlling the rate of flow was used to supply a constant flow of saline, at the rate of 15 drops per minute as an anticoagulant. The catheter was introduced into the median basilic vein of either arm. The passage of the catheter through the vein was accomplished while the patient was on a fluoroscopic table. There was no evidence of blood clotting or thrombi in the holes of the catheter.

Since the Cournand and Ranges (2) paper, venous catheterization has been employed to obtain blood samples from the coronary sinus of man (1, 12, 20), of the dog (8, 10, 11, 12, 13), the right auricle of man (2, 3, 4, 6, 12, 23, 24), of the dog (21), the hepatic vein of man (24), the portal vein of the dog (5), the jugular vein of man (18), of the dog (25), and the pulmonary artery of the dog (8, 10, 15), as well as in the measurement of cerebral blood flow in man (14), in the monkey (6, 22) and in the dog (14).

Catheters made of various materials have been used: steel or other metal cannulae (13, 14, 21), a soft ureteral catheter (9), a flexible radiopaque ureteral catheter (1, 2, 3, 4, 8, 10, 15, 24), and a modified, tapered tipped ureteral catheter with a woven, shellacked nylon core covered with a heavy X-ray-opaque

Received for publication June 20, 1949.

¹ Atomic Energy Commission postdoctoral research fellow in the Medical Sciences of the National Research Council.

plastic covering. This latter catheter has been modified (12) by the addition of two small side eyes, with shallow grooves on the outer surface of the catheter leading forward from the side eyes to the terminal eye. Unspecified plastic catheters (25), vinylite (18) and polyethylene (5) catheters have been used for intravenous work. Polyvinyl chloride tubing (19) was used for prolonged intramuscular injections.

Several thousand short-time catheterizations of the right auricle, to determine the cardiac output in man, have been performed in various laboratories without serious ill effects. However, it is a potentially dangerous procedure and should only be carried out with considerable care and thoughtfulness (20). Occasionally linear mural thrombosis occurred in patients near the site of incision when the catheters were left in place for 24 hours (3). Plastic intravenous catheters, used for protracted administration of various drugs to patients caused a transient phlebitis about the tube when left in the vein for several days (18). This condition may have been due to the injected fluids rather than the presence of the tube.

Dogs with the external jugular vein catheterized for 4 to 5 weeks showed that the veins were thrombosed around the tube in about half of the cases (25). In patients, the injection of materials into the median basilic vein for 12 days was satisfactory. This author felt that thrombosis of the veins occurred when concentrated or irritating substances were infused. Subendocardial fibrosis, mural thrombi and subendocardial hemorrhage were found in the right auricle after catheterization of the right heart (10). Following catheterization of the pulmonary artery, the right ventricle and the tricuspid valves showed some injury. Dogs were catheterized (11) seven times during 4 months, with only three failures out of 68 catheterizations. The catheters were left in the dogs up to 5 hours. Blood samples were obtained from the portal veins of dogs (5) for an average of 21 days and a maximum of 34 days after insertion of the catheter.

It is apparent from the foregoing summary that blood can be obtained satisfactorily by the use of catheters. Further investigations, however, are needed to improve the technique and to evaluate the effect of the infusion of various materials on long term blood sampling.

EXPERIMENTAL PROCEDURE

A smooth, flexible, transparent tubing made of polyvinyl chloride was used as a catheter. This tubing ("spaghetti") is used as insulation by electricians and can be obtained from electrical supply houses. Our catheters were 40 to 45 cm. in length with an inside diameter of 1.4 mm. and an outside diameter of 2.2 mm.

In our experiments catheters were placed in both external jugular veins of cows. Radioactive substances were injected into one jugular vein, while blood samples were drawn from the other. Prior to insertion of the catheter, the skin area over the jugular veins was closely clipped and local anesthetic (procaine) injected at the site of insertion. A jugular tourniquet was applied. Then a

sterile hypodermic needle of 10 to 12 gauge was thrust into the vein. A needle with a relatively short beveled point was used with the short edge of the bevel turned in the direction which the catheter was to travel. The sterile catheter was inserted through the needle into the vein, leaving 6 to 8 cm. protruding. The needle then was removed. A small clamp halted the flow of blood through the catheter and held the tube in place to prevent it from being drawn into the blood stream. Finally, the catheter was filled with a sodium heparin solution (4 mg. sodium heparin per 100 ml. of 0.9 per cent saline) to wash out the blood and prevent the formation of a clot. The catheter then was closed with a plug. A sterile, heavy silk suture was placed in the skin to form a loop close to the catheter. The catheter then was tied to the suture by making three single knots.

A syringe fitted with a 16 gauge needle was used to draw blood samples. To reduce the apprehension of the cow and to prevent her from pulling out the catheter during bleeding or injecting, the catheter was lengthened by using the tubing from a 16 gauge needle connected to an additional piece of "spaghetti" 15 to 20 cm. in length.

Prior to drawing a blood sample, the catheter was washed with heparinized saline to remove any clot which may have partially closed the tube. Occasionally considerable force was necessary to open the catheter. Often a valve-like clot formed in the catheter which permitted injection but not withdrawal. Following the bleeding, the injection of heparinized saline was repeated.

EXPERIMENTAL RESULTS

Nineteen catheters were used in twelve trials on five different cows. Blood was obtained through these catheters at the rate of 1 ml. per sec. Bleeding caused very little disturbance to the cows. One sample was secured while one cow was lying in a paddock chewing her cud. Numerous samples were drawn when the cows were eating or ruminating. The length of time a catheter remained opened varied from 2.1 hours to 14.5 days (table 1).

From one cow 24 samples were obtained in 14 hours. During a 38-day trial, another cow had five catheters inserted into the right jugular vein from which 135 samples of blood were taken. In the same period only one catheter was placed in the left jugular vein for repeated injections of radioactive material at 12-hour intervals.

Preliminary trials with calves as well as catheterization of the carotid artery of one dairy cow for 4 days show promise.

In two instances catheters were drawn into the blood stream, in one cow when the needle was being withdrawn and in the other cow when the tubing was being placed in the opposite vein. No deleterious physiological effects were observed during the following 66 and 48 days respectively, at which time the cows were slaughtered. At autopsy, one catheter was found in the right pulmonary artery. No gross abnormalities were observed.

Gresson and Glenn (5) report that polyethylene catheters caused a clotting

reaction in some of their dogs. Only in one out of twenty dogs did a complete thrombosis occur and then this followed three separate catheterizations of the portal vein at 2 and 3 week intervals, the catheter having been left in the vein

TABLE 1
Time venous catheters remained functional and number of samples obtained

Trial no.	Cow no.	Catheter	Blood samples per catheter	Functional time	Condition of catheter
1	798	a	4	3.3 hr.	occluded
2	890	a	4	2.1 hr.	occluded
3	798	a	9	15.6 hr.	open
4	890	a	6	1.1 d.	open
5	890	a	21	4.0 d.	open
6	798	a	17	1.0 d.	open
7	890	a	7	2.2 hr.	occluded
		b	10	3.8 d.	occluded
8	798	a	9	1.0 d.	open
9	890	a	13	3.0 d.	open
10	853	a	8	10.0 d.	occluded
11	834	a	8	3.1 d.	occluded
		b	25	4.3 d.	occluded
		c	43	8.5 d.	occluded
		d	35	7.0 d.	occluded
		e	24	14.5 d.	occluded
12	757	a	45	11.0 d.	occluded
		b	10	1.3 d.	occluded
		c	3	2.0 d.	occluded

for 2 months. In one of the cows, slaughtered 18 days after the catheters had been inserted, a complete thrombus was found in both the right and left jugular veins. The occluded portion corresponded approximately to the length of the catheters in the veins.

SUMMARY

A technique of venous catheterization has been adapted to dairy cows, which allows numerous blood samples to be taken at short intervals and the frequent, accurate intravenous injection of substances (particularly radioactive material) over a long period with a minimum of disturbance to the animals.

REFERENCES

- (1) BING, R. J., VANDAM, L. D., GREGOIRE, F., HANDELSMAN, J. C., GOODALE, W. T., AND ECKENHOFF, J. E. Catheterization of the Coronary Sinus and the Middle Cardiac Vein in Man. *Proc. Soc. Exptl. Biol. Med.*, **66**: 239-240. 1947.
- (2) Cournand, A., AND RANGES, A. Catheterization of the Right Auricle in Man. *Proc. Soc. Exptl. Biol. Med.*, **46**: 462-466. 1941.
- (3) Cournand, A., RILEY, R. L., BRADLEY, S. E., BREED, E. S., NOBLE, R. P., LAUSEN, H. D., GREGGENSEN, M. I., AND RICHARDS, D. W. Studies of the Circulation in Clinical Shock. *Surgery*, **13**: 964-995. 1943.
- (4) Cournand, A. Measurement of the Cardiac Output in Man Using the Right Heart Catheterization. *Federation Proc.*, **4**: 207-212. 1945.
- (5) CRESSON, S. L., AND GLENN, W. L. Venous Catheterization with Polyethylene Tubing. *J. Lab. Clin. Med.*, **33**: 1597-1602. 1948.

- (6) DEXTER, L. Venous Catheterization of the Heart. II. Results, Interpretations and Value. *Radiology*, 48: 451-462. 1947.
- (7) DUMKE, P. R., AND SCHMIDT, C. F. Quantitative Measurements of Cerebral Blood Flow in the Macaque Monkey. *Am. J. Physiol.*, 138: 421-431. 1942.
- (8) ECKENHOFF, J. E., HAFKENSCHIEL, J. H., FOLTZ, E. L., AND DRIVER, R. L. Influence of Hypotension on Coronary Blood Flow, Cardiac Work and Cardiac Efficiency. *Am. J. Physiol.*, 152: 545. 1948.
- (9) FORSSMAN, W. Die Sondierung des rechten Herzens. *Klinl. Wehnschr.*, 8: 2085-2087. 1929.
- (10) GOODALE, W. T., LUBIN, M., ECKENHOFF, J. E., HAFKENSCHIEL, J. H., DURLACHER, S. H., LANDING, B. H., AND BANFIELD, W. G. Coronary Sinus Catheterization Technique for Studying Coronary Blood Flow and Myocardial Metabolism in vivo. *Proc. Soc. Exptl. Biol. Med.*, 66: 571-573. 1947.
- (11) GOODALE, W. T., LUBIN, M., ECKENHOFF, J. E., HAFKENSCHIEL, J. H., AND BANFIELD, W. G., JR. Coronary Sinus Catheterization for Studying Coronary Blood Flow and Myocardial Metabolism. *Am. J. Physiol.*, 152: 340-355. 1948.
- (12) GOODALE, W. T., LUBIN, M., BANFIELD, W. G., JR., AND HACKEL, D. B. Catheterization of the Coronary Sinus, Right Heart and other Viscera with a Modified Venus Catheter. *Science*, 109: 117-119. 1949.
- (13) HARRISON, T. R., FRIEDMAN, B., AND RESNIK, H. Mechanism of Acute Experimental Heart Failure. *Arch. Int. Med.*, 57: 927-948. 1936.
- (14) KETY, S. S., AND SCHMIDT, C. F. The Determination of Cerebral Blood Flow in Man by the Use of Nitrous Oxide in Low Concentrations. *Am. J. Physiol.*, 143: 53-66, 1945.
- (15) KINNEY, T. D., HAYNES, F. W., AND DEXTER, L. Experimental Production of Pulmonary Embolism by the Use of a Venous Catheter. *J. Lab. Clin. Med.*, 30: 1013-1016. 1945.
- (16) KLEIBER, MAX., SMITH, A. H., AND RALSTON, N. P. Secretion in Cow's Milk of Intravenously Injected Radioactive Phosphorous P³². *Proc. Soc. Exptl. Biol. Med.*, 69: 354-356. 1948.
- (17) MEAD, D. J., TICHENOR, R. L., AND FLOSS, R. M. Electrical Properties of Solids. XII. Plasticised Polyvinyl Chloride. *J. Am. Chem. Soc.*, 64: 283-291. 1942.
- (18) MEYERS, L. Intravenous Catheterization. *Am. J. Nursing*, 45: 930-931. 1945.
- (19) MACQUIGG, R. E. New Techniques for Parenteral Fluid and Drug Administration: Muscular Intubation. *Surgery*, 18: 592-595. 1945.
- (20) POTTER, V. R. *Methods in Medical Research*. Vol. I. The Year Book Publishers, Inc. Chicago. 224-232. 1948.
- (21) SHORE, R., HOLT, J. P., AND KNOEFEL, P. K. Determination of Cardiac Output in the Dog by the Fick Procedure. *Am. J. Physiol.*, 143: 709-714. 1945.
- (22) SCHMIDT, C. F., KETY, S. S., AND PENNES, H. H. The Gaseous Metabolism of the Brain of the Monkey. *Am. J. Physiol.*, 143: 33-52. 1945.
- (23) SOSMAN, M. C. Venous Catheterization of the Heart. I. Indications, Techniques and Errors. *Radiology*, 48: 441-450. 1947.
- (24) WARREN, J. V., AND BRANNON, E. S. A Method of Obtaining Blood Samples Directly from the Hepatic Vein in Man. *Proc. Exptl. Biol. Med.*, 55: 144-146. 1944.
- (25) ZIMMERMAN, B. Intravenous Tubing for Parenteral Therapy. *Science*, 101: 567-568. 1945.

A METHOD FOR ESTIMATING THE FEED-REPLACEMENT VALUE OF PASTURE FORAGE

W. B. NEVENS, R. W. TOUCHBERRY AND J. A. PRESCOTT, JR.

Department of Dairy Science, University of Illinois, Urbana

Improved methods for determining the yields of pastures have been given much attention by both agronomic and livestock investigators. Agreement has been reached with regard to many of the procedures which should be followed and an outline of pasture techniques (1) has served as a useful guide for many research workers. An excellent review and appraisal of methods used in evaluating the results of pasture studies has been presented by Ahlgren (2).

The determination of yields by agronomic methods proves satisfactory under many conditions but has the limitation that yields alone do not indicate the value of the forage for livestock production. The investigation reported here-with had as its objective the designing of a simple method for the measurement of the feed-replacement value of pasture forage.

EXPERIMENTAL

Two experimental groups, each containing 13 registered Holstein cows, were established. Division into groups was made by selecting two cows as nearly alike as possible with respect to weight, stages of lactation and gestation, and level of milk yield, and then assigning at random one cow to the "Pasture Group" and the other to the "Dry-lot Group." The cows of the pasture group had been in milk for 109 days (group average) and those of the dry-lot group 116 days (group average) since the last calving date. Five cows of the pasture group were pregnant, the average stage of gestation being 31 days. One dry-lot cow had been pregnant for 22 days at the beginning of the trial.

The pasture group was allowed access to pasture and was given grain mixture (barn-fed) as the only supplementary feed. The dry-lot group was barn-fed on silage, alfalfa hay, moistened beet pulp and the same kind of grain mixture as that given to the pasture group. The silage feeding program was: corn silage (dry-matter content, 30.1 per cent) for 1 week; sorghum silage (dry-matter content, 22.3 per cent) for 5 weeks; corn silage (dry-matter content, 30.1 per cent) for 4 weeks; Sudan grass silage (dry-matter content, 29.3 per cent) for 4 weeks; and grass-legume silage (dry-matter content, 26.1 per cent) for 4 weeks. The alfalfa hay was chiefly second-cutting hay with a dry matter content of 84.2 per cent (average of determinations). The grain mixture consisted of ground corn, 350 lb.; ground oats, 300 lb.; wheat bran, 150 lb.; brewers' dried grains, 50 lb.; soybean meal, 120 lb.; bonemeal, 15 lb.; and salt 15 lb.

It originally was planned that the cows of the pasture group would be fed the same amount of grain mixture as the cows in the dry-lot group so that the amounts of feed saved by pasture could be measured in terms of roughages. It

Received for publication June 21, 1949.

was soon found, however, that the cows at pasture would not eat as much grain mixture as the ones in the dry-lot group.

The experimental period extended from May 17 to September 19, 1948, inclusive, a total of 126 days. Both groups of cows were started on experimental feeding at the same time and were carried throughout the entire period without change. After the cows had been on trial for 1 week and had become adjusted to the experimental feeding, they were weighed for 3 successive days. They also were weighed on 3 successive days at monthly intervals and at the end of the trial period.

The amounts of feed (other than pasture forage) which were offered and refused were carefully weighed and recorded. The amount of milk produced by each cow at each milking was weighed and butterfat tests were made of composite samples of milk made up from the milkings of each cow on 4 days of each week. Production records were computed to an F.C.M. (fat-corrected milk) basis (3).

The pasture consisted of grass-legume mixtures seeded in the spring of 1947 and grazed for the first time at the beginning of this trial. The crops included bromegrass-Ladino clover, bromegrass-alfalfa, bromegrass-birdsfoot trefoil and orchard grass-Ladino clover. Rotational grazing was practiced for a part of the season. The dry-matter yield of the pasture forage as determined by the protected-cage and clipping method was 9,370 lb. per acre, of which 1,320 lb. remained on the pasture at the close of the trial.

RESULTS

A summary of the feed consumption, milk production, and liveweight gains is given in table 1. The average daily milk production during the trial was

TABLE 1

Milk production, liveweight gains and feed consumption of cows kept in dry lot and at pasture

	Dry lot group		Pasture group		Feed saved per acre of pasture ^a
	Per cow daily	Per 100 lb. F.C.M.	Per cow daily	Per 100 lb. F.C.M.	
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
F.C.M. produced	34.1		35.1		
Liveweight gain	0.8		1.3		
Feed consumed:					
Silage	31.9	93.8			3,739
Hay	17.2	50.6			2,017
Beet pulp	1.5	4.4			175
Grain mixture	11.9	34.9	7.8	22.1	510

^a Or feed-equivalent value per acre of pasture.

practically the same for each group. The cows in the pasture group made slightly greater liveweight gains than the dry-lot cows, thus showing that the pastured cows were as well fed as those in the dry-lot group.

The amount of feed saved by pasture is found from table 1 by subtracting the amounts of feed other than pasture forage consumed by the pasture group from the amounts eaten by the dry-lot group. For each 100 lb. of F.C.M. it was 93.8 lb. of silage, 50.6 lb. of hay, 4.4 lb. of dried beet pulp, and 12.8 lb. of grain mixture. These amounts multiplied by the yield of F.C.M. per acre (39.86 cwt.) gives 3,739 lb. of silage, 2,017 lb. of hay, 175 lb. of beet pulp and 510 lb. of grain mixture, respectively, as the feed saved, or the feed-equivalent value, of an acre of pasture (table 1).

The figures also furnish the basis for calculating the money value of an acre of pasture. The receipts from the sale of milk produced per acre (3,986 pounds F.C.M.) less the cost of the grain mixture fed per acre (881 lb.) gives the cash value of the feed obtained from pasture.

ANALYSIS AND DISCUSSION OF DATA

The yield of F.C.M. in pounds for each cow was calculated for each of the 18 weeks. The yields of one pair of animals for 1 week were called a weekly pair. Table 2 gives the analysis of variance of the weekly pairs on the basis of the variation between and within weekly pairs.

TABLE 2
Analysis of variance of F.C.M. per cow per week

Source	Sum of squares	Degrees of freedom	Mean square
Total	2,435,835	467	
A—Between weekly pairs	1,711,064	233	
Weeks	520,846	17	30,638.00**
Cow pairs	1,085,634	12	90,469.50**
Weeks × cow pairs	104,584	204	512.67
B—Within weekly pairs	724,771	234	
Rations	6,030	1	6,030.00
Rations × weeks	24,965	17	1,468.53
Rations × cow pairs	506,998	12	42,249.83**
Rations × weeks × cow pairs	186,778	204	915.58

** Significant at the 0.01 level of probability.

The sum of squares between weekly pairs was broken down into three parts. One part was associated with the differences between cow pairs. Another was associated with the differences brought about by the time trend as measured in weeks. The third part was associated with the interaction of the two former sources. Using the mean square for interaction between weeks and cow pairs as an error term, the mean squares for weeks and cow pairs were significant at the 0.01 level of probability. This was expected and hardly needs an explanation.

The sum of squares within weekly pairs was broken down into four parts. These four parts were associated with the differences between rations and the two first-order interactions and the one second-order interaction involving rations.

Using the second-order interaction, ration \times weeks \times cow pairs, as an error term, the two first-order interactions were tested. The interaction, ration \times cow pairs, was significant at the 0.01 level of probability. This may indicate a differential rate of response to the two rations from one pair of cows as compared to another. It may also be a fact that the members of a pair just failed to perform similarly irrespective of the ration fed. The intraclass correlation of the weekly pairs was 0.407, which shows that the pair members were far from being identical.

The first-order interaction, rations \times weeks, was not significant. This points out that one ration was as consistent as the other in maintaining F.C.M. yield for the 18 weeks. There was no appreciable differential response from the two rations from one week to another. In figure 1 the regression of F.C.M.

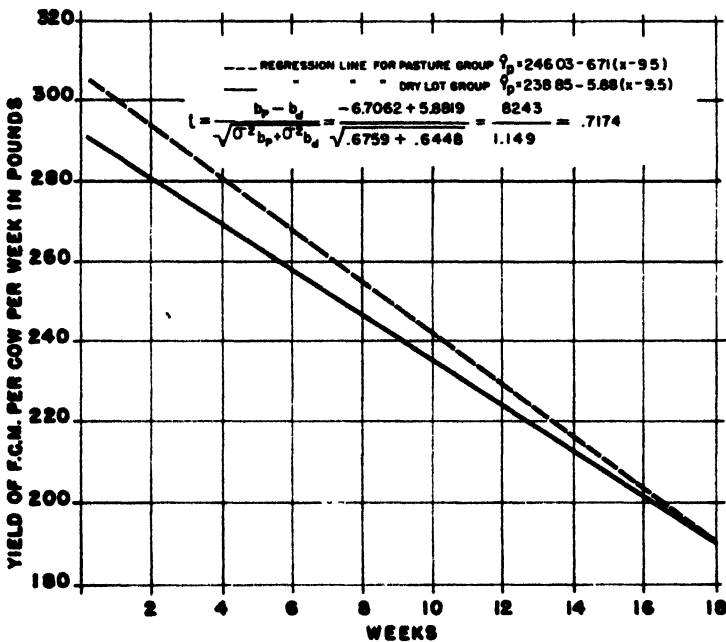


FIG. 1. The regression of F.C.M. per cow per week on time as measured in weeks.

per cow per week on weeks for the pasture group is slightly larger than the corresponding regression for the dry-lot group. Both regression coefficients are negative, and are not significantly different from each other.

The mean square for rations when tested by the interaction, ration \times cow pairs, was not significant. Since the mean square for ration was smaller than that for the ration \times cow pair interaction, the interaction was divided by the main effect in making the F test. From the analysis of variance and regression lines one can say that the two rations have not produced significantly different effects on the yield of F.C.M.

Table 3 gives the analysis of variance for the monthly weights of the cows on experiment. The weight at each interval or month was recorded as the average of three successive daily weights. The sum of squares for weights was broken down into the two parts, between monthly pairs and within monthly pairs. The differences associated with months and cow pairs were highly significant. The difference between the weights of the cow pairs was expected because of the pairing of similar animals. The changes in weight from one month to the next were mainly a manifestation of gain in weight. Figure 2 shows that in only

TABLE 3
Analysis of variance of the monthly weights

Source	Degrees of freedom	Sum of squares	Mean square
Total	129	2,012,315	
Between monthly pairs	64	1,245,752	
Cow pairs	12	931,829	77,652**
Months	4	284,871	71,218**
Months \times cow pairs	48	29,052	605
Within monthly pairs	65	766,563	
Rations	1	2,669	2,669
Rations \times months	4	18,405	4,601*
Rations \times cow pairs	12	681,117	56,760**
Rations \times months \times cow pairs	48	64,372	1,341

* Significant at 0.05 level of probability.

** Significant at 0.01 level of probability.

one case was there a loss in weight, and this was during the second month. The cause of this loss of weight is not known.

The sum of squares within monthly pairs was broken down into four parts. That part attributed to differences between rations was not significant while the first order interaction mean squares rations \times months and rations \times cow pairs were significant at the 0.05 and 0.01 level of probability, respectively. The ration \times cow pairs interaction is an expression of a differential response of the cow pairs to the two rations; this may be nothing more than the failure of members of pairs to perform similarly in changes in weight irrespective of ration. The ration \times months mean square denotes a differential response to the two rations from one month to another. Figure 2 gives the average weight of the two groups at monthly intervals, and the interaction is clearly shown in the third and fourth monthly periods. The regressions of weight on time as measured in monthly intervals (fig. 2) were not significantly different for the two rations. A *t* test of the gains of the pair members showed no difference in the total gains made under each ration.

The analyses made make it possible to accept the hypothesis that there was no difference in the F.C.M. yield and weight gains of the two groups on the two different rations. Approximately the same amount of energy was consumed by the two groups, so the pasture has replaced the amount of grain, hay, and silage

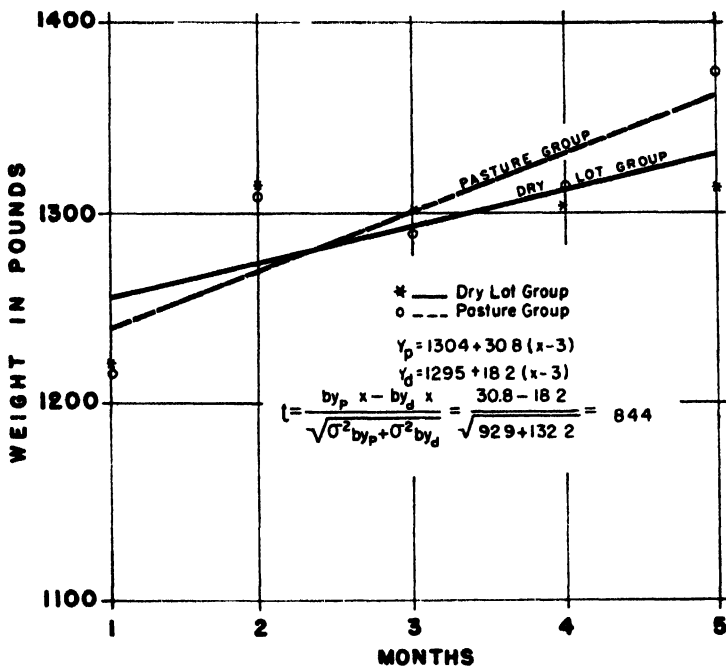


FIG. 2. Regression of weight in pounds as measured in monthly intervals.

which the cows on dry lot had in excess to that given the pasture group. This is probably a minimum estimate of the replacement value of pasture, because of more exercise of the pasture group, and because of the slightly larger, but not significantly so, yields of F.C.M. and gains in weight of the pasture group.

SUMMARY AND CONCLUSIONS

The milk-production value of pasture in terms of the amount of feed replaced by pasture forage was studied by means of a group of 13 cows fed only grain mixture and given access to pasture and a comparable group of 13 cows kept continuously in dry lot and barn fed on silage, hay, beet pulp and grain mixture over a period of 18 weeks. The amounts of milk produced were large and were practically the same for each of the two groups throughout the experimental period. Live weight gains for both groups were good, indicating that the amounts of nutrients supplied were ample.

Statistical analyses of the data indicate that there were no significant differences in the F.C.M. yield and weight gains of the two groups on the different rations. The feed-equivalent value of an acre of the pasture used in this investigation was found to be 3,739 lb. of silage, 2,017 lb. of hay, 175 lb. of beet pulp and 510 lb. of grain mixture.

The procedures followed provide a dependable method for estimating the value of pasture forage consumed by dairy cows.

REFERENCES

- (1) Anon. Preliminary Report of Pasture Investigations Committee. J. Dairy Sci., 26: 353-369. 1943.
- (2) AHLGREN, H. L. A Comparison of Methods Used in Evaluating the Results of Research. J. Amer. Soc. Agron., 39: 240-259. 1947.
- (3) GAINES, W. L. The Energy Basis of Measuring Milk Yield in Dairy Cows. Ill. Agr. Expt. Sta. Bull. 308. 1928.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

NOVEMBER, 1949

NUMBER 11

VARIOUS CARBOHYDRATES AS ENERGY SOURCES FOR SOME MIXED CULTURES OF SILAGE ORGANISMS^{1,2}

R. L. SALSBUURY,³ R. E. MATHER AND C. B. BENDER

New Jersey Agricultural Experiment Station, Sussex

Much of the work on the bacteriology of silage has been concerned with the isolation and identification of pure cultures of organisms which supposedly predominate in the silage and with the determination of total counts at various stages of the fermentation. For practical purposes, however, the primary interest is in the effect of the bacterial population of the silage, a population that is heterogeneous and may vary in composition with each silage made, and even from sample to sample, but which gives, ultimately, the same result in a large number of cases. Hence, a study using a mixed culture, which actually represents the bacterial population of the silage at the time each sample is taken, might furnish information which could not be supplied by work with pure cultures.

The desired effect in silage preservation is acid production by lactic acid bacteria from carbohydrates. The relative utilization of various carbohydrates, as measured by titration of the acid produced, is the basis for the classification of these organisms by Orla-Jensen (1). The pH attained by cultures also may be used to evaluate utilization of carbohydrates (2, 3).

The present experiment was designed to test the preference of the bacterial population, as it exists in the silo, for a number of carbohydrates. It was thought that a change in the predominating type of organism or in the carbohydrate available at a given time might be reflected in the degree to which the different carbohydrates were utilized under standard conditions.

EXPERIMENTAL

Two silages, put up in 1946 and 1947, respectively, were used for this experiment. The material was chopped in the field and, in 1946, chopped again as it was being put into the silo. The crop ensiled in 1946 consisted of alfalfa, red clover and grass, to which was added about 70 lb. of molasses per ton of green material. In 1947 the crop ensiled was principally reed canary grass with a

Received for publication May 23, 1949.

¹ Paper of the Journal Series—New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, Department of Dairy Industry.

² This project was supported by a research grant from the Sugar Research Foundation, Inc., New York, N. Y.

³ Present address: Michigan Department of Health, Lansing.

small amount of red clover. Molasses was added at the rate of approximately 180 lb. per ton of green material in the lower part of the silo; in the upper part, 100 lb. was added per ton. Samples of the mixture were taken from the surface just after it had been blown into the silo when the level reached the 10-ft. mark and again at the 20-ft. mark. Subsequent samples were obtained by drilling a hole in the wall of the silo, removing the sample and plugging the hole. The settling of the silage at each level was measured by means of a float connected by wire over pulleys with an indicating weight on the outside of the silo. Allowance for settling was made when boring the hole for sampling in an attempt to reduce variation which might be attributable to heterogeneous crop mixtures and molasses dispersion.

Determinations for pH were made with a Beckman pH meter on juice expressed from the silage by a small press. Dry matter determinations were made by the toluene distillation method.

For the bacteriological work, a 10-g. sample was placed in a 90-ml. sterile water blank. After being shaken, 1 ml. of this suspension was placed in a 99-ml. sterile water blank. Culture tubes containing 9 ml. of the differential media then were inoculated with 1-ml. portions of the final dilution. These were incubated for 4 days at 30° C. and the acid produced was titrated with 0.1 N NaOH, using phenolphthalein as the indicator. The titration value of the uninoculated medium was subtracted from the value obtained. The medium contained 0.5 per cent Difco yeast extract, 0.2 per cent potassium dihydrogen phosphate, 0.1 per cent magnesium sulfate, 0.04 per cent sodium chloride and 2.0 per cent carbohydrate. The carbohydrates used were xylose, arabinose, rhamnose, fructose, glucose, galactose, sucrose, lactose, raffinose, inulin, dextrin, soluble starch, glycerol, mannitol, sorbitol and salicin.

Analyses of variance and covariance were used to test the differences between crops, silo levels (and molasses addition levels) and among carbohydrates. Interrelationships among the carbohydrates, days after ensiling, pH and dry matter were studied by partial correlations calculated on a within carbohydrate, within year-level lot basis.

RESULTS AND DISCUSSION

Acidity and dry matter. The development of acidity (as measured by pH) and the dry matter content of the various samples of silage are illustrated in table 1, together with the acid production of the cultures in terms of ml. of 0.1 N acid as an average for all 16 carbohydrates. In all four lots the pH was reduced to 4.54 or less by the third day and 4.32 or less by the seventh day, indicating satisfactory acid development. The dry matter content of the silages was somewhat higher and the development of acidity a little slower in 1947 than in 1946, in spite of addition of more molasses in 1947. The average linear regression of pH on days in silo was significant, as indicated by the correlation -0.374, but the correlation between pH and log days was considerably higher (-0.729). A prediction equation $\text{pH} = 4.600 - 0.318 d'$ (where $d' = \log \text{days in silo}$) yielded estimated pH values for 0, 1, 2, 8 and 32 days of 5.24, 4.60, 4.50, 4.31 and 4.12, respectively.

The 1946 silage, with lower dry matter content and more legumes, had a sweeter odor and appeared to be more palatable and better than that of 1947.

Fermentation studies. The over-all mean values for the 16 carbohydrates studied (table 2) were shown to differ significantly by the analysis of variance. The differences between years and between levels were very small and not statistically significant, and the lack of a significant interaction between carbohydrate and year-level lot prevents the consideration of changes in differences between carbohydrates from one lot to another.

Correlation coefficients calculated within lot from the data of table 1 indicated no relationship between dry matter content of the sample and the amount of acid produced by the cultures.

TABLE 2

Average amount of acid (as ml. 0.1 N acid) produced by mixed cultures of silage organisms after incubation for 4 days at 30° C. for each of 16 carbohydrates

Group	Sugar	ML. 0.1 N NaOH	Rank
Pentoses	Xylose	3.30	5
	Arabinose	4.68	1
Methyl pentose	Rhamnose	1.07	15
Hexoses	Fructose	3.47	4
	Glucose	4.05	2
	Galactose	2.46	8
	Sucrose	3.93	3
Disaccharides	Lactose	2.34	9
	Raffinose	2.51	7
Trisaccharide	Inulin	1.69	12
	Dextrin	1.72	11
	Soluble starch	2.22	10
	Glycerol	.88	16
Alcohols	Mannitol	1.49	13
	Sorbitol	1.42	14
	Salicin	3.04	6

The longer the material remained in the silo, the lower was the acid production of the cultures, as indicated by correlations of -0.438^{**} between mean acid production and days in silo and -0.451^{***} between logarithm of mean acid produced and log days. Thus the curvilinear relationship is very slight.

A correlation between mean and standard deviation indicated the desirability of transforming all acid production values to logarithms, but several negative values prevented the use of logarithms throughout. For six of the sugars, fructose, glucose, galactose, sucrose, lactose and raffinose, the within sugar within lot correlation between days in the silo and log acid production was -0.229^{**} (191 degrees of freedom). Similarly, the within sugar within lot correlation for these six sugars between pH and log acid production was 0.238^{**} .

Simple correlation coefficients among the acid production values of the six sugars previously mentioned and the correlation of these values with days in the

* Throughout this paper * represents significance at the 5 per cent point and ** represents significance at the 1 per cent point.

silo and with pH of the silage sample are presented in table 3. In general, the correlations are not high, but five are highly significant. The general activity of the cultures, as related to days in the silo and to the pH of the silage samples, undoubtedly would influence the size of the correlations among the acid production values of the six sugars. Partial correlation coefficients, independent of these two relationships and also independent of fluctuations in the most closely related sugars, were lower than most of the values of table 3, but in other cases very different relationships appeared. The most pronounced change in correlation was observed in the relationships between glucose and raffinose where the simple correlation was -0.198 ; when independent of fluctuations associated with days in the silo and fructose and galactose, the correlation was -0.588^{**} but when independent of pH, fructose and galactose it was only -0.051 .

These results, though based on rather limited data, indicate that there is comparatively little systematic change in the preference of the mixed cultures of silage organisms as the fermentation progresses, while there is a definite decrease in the amount of acid produced by the mixed cultures the longer the silage has been fermenting, which undoubtedly is a function of the number of viable organisms present.

TABLE 3

Correlation coefficients among acid production values (logarithms) of six sugars, age of silage and pH of silage, within lot

	Fructose 4	Glucose 5	Galactose 6	Sucrose 7	Lactose 8	Raffinose 9
d Days	-0.033	-0.242	-0.200	-0.215	-0.451**	-0.151
p pH	0.210	0.299	0.211	0.329	0.295	0.156
4 Fructose		0.300	0.657**	0.621**		0.246
5 Glucose			0.573**	0.399*	0.048	-0.198
6 Galactose					0.042	0.382*
7 Sucrose					0.153	0.354*
8 Lactose						0.506**

The most desirable carbohydrates for silage preservation from the standpoint of rapid acid production would seem to be arabinose, glucose, sucrose, fructose and xylose. However, it must be kept in mind that since molasses was used as a preservative for the silage studied here, the organisms which developed most rapidly undoubtedly were the ones which could utilize the sugars of molasses most efficiently. The polysaccharides produced less acid than the sugars, indicating one reason why greater quantities of cereal grains than molasses may be required for silage preservation. Likewise, the comparatively low acid production of lactose explains why dried whey is a less desirable preservative than molasses.

In addition to consideration of the various carbohydrates as preservative materials to add to silage, these results reemphasize the importance of ensiling a crop when it is high in simple sugars, rather than when it becomes so mature that a large proportion of the carbohydrates in the plant are present as polysaccharides. In some cases, of course, this is counterbalanced by the much larger total amount of carbohydrate present in more mature plants.

SUMMARY AND CONCLUSIONS

A study was made to determine the relative amounts of acid produced by mixed cultures of silage organisms when offered various carbohydrates as sources of energy.

As the silage aged, the viability of the cultures was somewhat reduced, though considerable variation was observed with some carbohydrates. This decrease did not follow the pH of the silage samples much more closely than it followed a simple linear decline with age.

Interrelationships among six sugars, in terms of fluctuations of the logarithms of acid production about the mean or regression on age or on pH of the silage, showed that adjustment for pH of the sample reduced most correlations, though correlations between variations of glucose and galactose, fructose and galactose, and sucrose and levulose independent of glucose remained highly significant.

Arabinose, glucose, sucrose, fructose and xylose resulted in the production of more acid than the other carbohydrates used. There were no significant changes in acid production in the 2 years studied even though the nature of the crop and the amount of molasses added as a preservative changed considerably.

REFERENCES

- (1) ORLA-JENSEN, S. The Lactic Acid Bacteria. Andr. Fred. Høst and Son. Copenhagen. 1919.
- (2) PEDERSON, C. S. A Study of the Species *Lactobacillus plantarum* (Orla-Jensen). Bergey *et al.* J. Bact., 31: 217-224. 1936.
- (3) PEDERSON, C. S. The Gas-Producing Species of the Genus *Lactobacillus*. J. Bact., 35: 95-108. 1938.

THE NUTRITIVE VALUE OF WOOD MOLASSES AS COMPARED WITH CANE MOLASSES¹

N. F. COLOVOS, H. A. KEENER, J. R. PRESCOTT AND A. E. TEERI²

New Hampshire Agricultural Experiment Station, Durham

One of the many objectives of the Forest Products Laboratory of the U. S. Forest Service at Madison, Wis., is to find and develop new uses for low quality wood and wood wastes. One such development of this laboratory is the production of wood molasses.

Using a dilute acid process for hydrolysis similar to one used in Germany with many modifications as reported by Harris (6), yields of 130 to 200 gallons of molasses containing 50 per cent sugar are obtained from each ton of wood. The process consists of hydrolyzing the cellulose of waste wood to form glucose by means of dilute sulfuric acid and increased temperature and pressure. After neutralization, the resulting solution is concentrated to form molasses.

Although wood molasses has many potential uses in industry, it also has very important possibilities as a feed for livestock. Molasses made from wood was fed to farm animals as early as World War I. It was tested experimentally as a feed for livestock in Germany and certain Scandinavian countries during the recent war. While wood molasses has been reported to be an acceptable feed for livestock as a result of preliminary feeding trials conducted in this country (7), few quantitative measurements appear to have been made with farm animals to determine its nutritive value.

Because wood molasses might be used in livestock feeding in the same manner as cane molasses and because the nutritive properties of this latter product are very well known, these two products were compared by means of balance experiments with dairy heifers.

EXPERIMENTAL

The wood molasses fed in this experiment was made from pine wood wastes at the Forest Products Laboratory, Madison, Wis. It was compared with cane molasses purchased from a local feed store. The hay with which the molasses was fed was a field-cured grass-legume mixture from a lot part of which had been fed as the sole ration in unreported nitrogen and energy balance experiments.

The animals used in all of the balance experiments were purebred dairy heifers weighing between 630 and 900 lb. at the beginning of the experiment and included four Guernseys, one Ayrshire and one Holstein. Each animal was fed a daily basal maintenance ration of 6 kg. of hay on all balances with the exception of one small heifer, no. 6, which received 5 kg. Two kg. of wood molasses or its energy equivalent of cane molasses were added to the basal hay ration of each animal in

Received for publication June 13, 1949.

¹ Scientific Contribution no. 128 of the New Hampshire Agricultural Experiment Station.

² The authors gratefully acknowledge the cooperation of A. D. Littlehale of the staff of this Station under whose careful supervision the animals were kept.

the molasses balances with the exception of heifer no. 6, where the amount of molasses fed was reduced in proportion to the hay. All of the balances were carried out between October 13, 1948, and March 12, 1949, with the animals being allotted to the various rations according to table 1.

The procedure and methods followed in this experiment were essentially the same as those used in previous research reported from this laboratory (2), with some modifications described in a recent paper (4). The molasses was mixed thoroughly with the chopped hay before feeding. Because of the lumpiness of the wood molasses, a daily aliquot was taken for each animal during the collection period to insure a representative sample. However, the method of production has been perfected recently so that wood molasses of uniform consistency can be produced. The cane molasses was more homogeneous so a representative sample was taken from the drum after thorough mixing. The molasses samples were analyzed in triplicate for both nitrogen and gross energy, the samples used for the gross energy determination first having been dried in the capsules under vacuum.

TABLE 1
Schedule of balance experiments

Heifer no.	Breed	Hay balances	First molasses balance	Second molasses balance
1	Guernsey	Hay		
2	Guernsey	Hay	Hay + wood molasses	
4	Holstein	Hay	Hay + cane molasses	Hay + wood molasses
3	Guernsey		Hay + wood molasses	Hay + cane molasses
5	Ayrshire		Hay + cane molasses	Hay + wood molasses
6	Guernsey		Hay + wood molasses	Hay + cane molasses

The preliminary feeding periods were of about 7 days duration, while the collection periods were from 14 to 18 days in length. Three consecutive 12-hr. heat production measurements were made at the end of each collection period by means of the open circuit respiration chamber.

RESULTS AND DISCUSSION

The nitrogen content of the wood molasses calculated as protein was 0.44 per cent while that of the cane molasses was 2.83 per cent. The gross energy on the other hand was higher in the wood molasses. This necessitated feeding a little more cane molasses in order to have the intake of energy the same from both kinds of molasses.

The protein and energy contents of the hay, cane molasses and the individual composite samples of wood molasses are given in table 2. Summaries of the nitrogen balances are given in table 3, energy balances in table 4 and metabolizable energy values in table 5.

Although it was desired to feed the basal ration of hay at as near the maintenance level as possible, a little extra was fed in order to avoid the possibility of submaintenance feeding of the growing heifers. This resulted in an average gain of 8.109 g. of nitrogen and 821 Calories of energy per day on the hay ration. The

retention of nitrogen was increased when both the molasses supplements were fed, even though in the case of the wood molasses relatively little additional nitrogen was ingested. This effect appears to have been due to an inadequate energy intake from non-nitrogenous sources on the basal hay ration. Digestibility of nitrogen, on the other hand, was depressed with the addition of both the wood and cane molasses to the hay. The apparent digestibility of the nitrogen in the wood molasses-plus-hay ration was 12.1 per cent less than that of hay alone. The addition of the cane molasses to the hay lowered the apparent digestibility of the nitrogen by 4.8 per cent.

While there are no reports in the literature on the effect of wood molasses on the apparent digestibility of the ration, Lindsey and Smith (9), Williams (10), Briggs and Heller (3), Hamilton (5), as well as others, found that the addition of cane molasses to the ration lowered the apparent digestibility of the nitrogen to varying degrees. The probable explanation for this decrease is that the increased dry matter intake resulted in a higher metabolic nitrogen excretion. This explanation is supported by the work of Armsby (1), and of Harris and Mitchell

TABLE 2
Composition of feeds

Feed	Moisture	Protein	Gross energy
	(%)	(%)	(Cal./g. D.M.)
Hay	9.19	10.644	4.3102
Cane molasses	26.86	2.825	3.6608
Wood molasses fed to:			
Heifer 2	23.67	0.369	3.8793
Heifer 3	23.89	0.288	3.9761
Heifer 4	25.82	0.406	4.0358
Heifer 5	27.10	0.519	4.0561
Heifer 6	26.42	0.613	4.0204
Av.	25.38	0.439	3.9935

(8), which showed that when molasses was added to nitrogen-free rations being fed to sheep, there was an increase in the metabolic nitrogen. Using the factor of 5.5 mg. for metabolic nitrogen in the feces per gram of dry food consumed, as worked out by Harris and Mitchell (8), the average true digestibilities of the protein of the basal hay ration, the wood molasses-plus-hay and the cane molasses-plus-hay rations are essentially the same, being 82.4, 79.4, and 83.0 per cent, respectively. Thus, the significantly lower apparent digestibility of the nitrogen in the two molasses-plus-hay rations, as compared with that of the basal hay ration, is almost entirely accounted for by the increase in fecal nitrogen resulting from the higher dry matter intake.

The retention of energy was increased markedly when both the wood and cane molasses supplements were fed. The average digestibility of the energy increased 2.4 and 3.2 per cent, respectively, when the wood and the cane molasses were fed, but this difference was not significant. The metabolizable energy increased about the same amount when both the wood and cane molasses were added to the hay ration. The metabolizability percentage of the two molasses rations was essentially the same and not significantly higher than that of the basal hay ration.

TABLE 3
Average daily nitrogen balances

Ration	Hay			Hay + wood molasses						Hay + cane molasses					
	1	2	4	2	3	4	5	6		3	4	5	6		
Heifer no.															
Body weight (lb.)	690	715	715	739	724	798	908	631		745	763	872	645		
Intake (g.)	102.180	102.180	102.180	93.272	100.445	103.480	103.840	86.777		112.124	112.124	112.124	94.190		
Outgo (g.)															
Feces	48.735	47.178	47.736	54.743	56.907	61.904	61.241	52.644		58.607	57.619	56.469	49.551		
Urine	49.301	43.334	45.975	34.888	23.868	31.299	33.262	21.658		42.943	32.984	48.342	27.922		
Total	98.036	90.512	93.711	89.631	80.865	93.203	94.503	74.302		101.550	90.603	114.811	77.473		
Balance (g.)	+4.144	+11.668	+8.469	+13.729	+19.580	+10.277	+9.337	+12.475		+10.574	+21.521	+7.313	+16.717		
Digestibility (%)	52.30	53.53	53.28	41.31	43.25	40.18	41.02	39.34		47.73	48.60	49.64	47.39		
Average balance (g.)		8.09				13.09						14.03			
Average digestibility ^a		53.14				41.02						48.34			

^a All differences are significant at the 1 per cent level.

TABLE 4
Average daily energy balances

Ration	Hay			Hay + wood molasses			Hay + cane molasses					
	1	2	4	2	3	4	5	6	3	4	5	6
Heifer no.												
Body weight (lb.)	690	715	715	739	724	798	908	631	745	763	872	645
Intake (Cal.)	23485	23485	23485	29407	29537	29473	29399	24481	29376	29376	29376	24925
Output (Cal.)												
Feces	9085	9590	9901	11097	11267	11109	11169	9740	10706	11261	11098	9121
Urine	933	817	826	1041	813	1029	1008	753	953	956	1053	823
Methane	1507	1332	1452	1709	1900	1899	1731	1453	1753	1930	1755	1519
Heat production	10802	11442	10304	13125	11494	12086	12791	9995	11497	11959	11538	10116
Total	22327	23181	22483	26972	25474	26123	26699	21941	24909	26106	25444	21579
Balance (Cal.)	+ 1158	+ 304	+ 1002	+ 2435	+ 4063	+ 3350	+ 2700	+ 2540	+ 4467	+ 3270	+ 3932	+ 3346
Digestibility (%)	61.3	59.2	57.8	62.3	61.9	62.3	62.0	60.2	63.5	61.7	62.2	63.4
Average energy balances (Cal.)		821				3050					3754	
Average digestibility ^a		59.4				61.8					62.6	

^a Differences in digestibility are not significant.

TABLE 5
Average daily metabolizable energy

Ration	Hay			Hay + wood molasses			Hay + cane molasses		
	1	2	4	2	3	4	5	6	6
Heifer no.									
Body weight (lb.)	690	715	715	739	724	798	908	631	645
Dry matter consumed (g.)	5449	5449	5449	6973	6971	6933	6907	5762	6003
Gross energy (Cal.)	23485	23485	23485	29407	29537	29473	29399	24481	24925
Metabolizable energy (Cal.)	11939	11659	11243	15458	15411	15359	15421	12423	13365
Metabolizable energy/ g. dry matter (Cal.)	2.191	2.140	2.063	2.217	2.211	2.215	2.233	2.329	2.226
Metabolizability (%)	50.8	49.6	47.9	52.6	52.2	52.1	52.5	50.7	53.6
Average metabolizable energy/g. dry matter (Cal.)		2.131				2.241			
Average metabolizability ^a		49.4				52.0			
							2.201		
							52.9		

^a Differences in metabolizability percentage are not significant.

When calculated by difference, the metabolizable energy values, expressed as Cal. per g. dry matter, were found to be 2.537 for the wood molasses and 2.403 for the cane molasses.

SUMMARY AND CONCLUSIONS

The nutritive value of wood molasses was compared with cane molasses by means of 12 protein and energy digestion balance experiments with dairy heifers. Both the cane and wood molasses contained very little protein although the cane molasses contained 7 times more protein than the wood molasses. The wood molasses excelled the cane molasses in gross energy content by about 10 per cent on the dry basis.

The apparent digestibility of the protein in the ration was depressed when both the cane and wood molasses were added to the basal hay ration. When cane molasses was added, the decrease was 4.8 per cent, while with the addition of the wood molasses the digestibility of the protein was decreased by 12.1 per cent. However, almost all of this effect can be accounted for on the basis of increased metabolic nitrogen excretion in the feces. The digestibility as well as metabolizability of the energy was greater when both the wood and cane molasses were added to the basal hay ration but the differences were not significant. The metabolizable energy per gram of dry matter was essentially the same in both molasses.

The results of this experiment indicate that wood molasses is comparable to cane molasses as a feed for dairy cattle.

REFERENCES

- (1) ARMSBY, H. P. *The Nutrition of Farm Animals*. The Macmillan Co., New York. P. 619. 1917.
- (2) BENEDICT, F. G., COROPATCHINSKY, V., AND RITZMAN, E. G. Technik der Messung des Gesamtstoffwechsels und des Energiebedarfes von Haustieren. *Abderhalden's Handb. d. biolog. Arbeitsmethoden, Abt. IV, Teil 13*: 619-687. 1934.
- (3) BRIGGS, H. M., AND HELLER, V. G. The Effect of Adding Blackstrap Molasses to a Lamb Fattening Ration. *J. Agr. Research*, 60: 65-72. 1940.
- (4) COLOVOS, N. F., KEENER, H. A., PRESCOTT, J. R., AND TEERI, A. E. The Nutritive Value of Timothy Hay at Three Different Stages of Maturity Compared with Second Cutting Clover Hay. *J. Dairy Sci.*, 32: 659-664. 1949.
- (5) HAMILTON, T. S. The Effect of Added Glucose Upon the Digestibility of Protein and of Fiber in Rations for Sheep. *J. Nutrition*, 23: 101-110. 1942.
- (6) HARRIS, E. E. Wood-sugar Molasses from Wood Waste. U.S.D.A. Forest Service, Forest Products Laboratory, Madison, Wis. Publ. no. R1704. 1947.
- (7) HARRIS, E. E. Animal Feeds from Wood Residue. *Forest Products Research Soc. Rept.* Pp. 1-5. 1948.
- (8) HARRIS, L. E., AND MITCHELL, H. H. The Value of Urea in the Synthesis of Protein in the Paunch. *J. Nutrition*, 22: 167-182. 1941.
- (9) LINDSEY, J. B., AND SMITH, P. H. Effect of Porto Rico Molasses on Digestibility of Hay and Concentrates. *Mass. Agr. Expt. Sta. Ann. Rept.*, 22: 82-131. 1909.
- (10) WILLIAMS, P. S. The Effect of Cane Molasses on the Digestibility of a Complete Ration Fed to Dairy Cows. *J. Dairy Sci.*, 8: 94-104. 1925.

THE EFFECT OF VACCINATION WITH BRUCELLA ABORTUS VACCINE (STRAIN 19) ON CERTAIN BLOOD CONSTITUENTS IN YOUNG HEIFERS¹

NORMAN S. LUNDQUIST,² C. ERNEST ZEHNER AND PAUL H. PHILLIPS

Department of Dairy Husbandry and Biochemistry College of Agriculture, University of Madison, Wisconsin

The development in young calves of immunity to *Brucella abortus* by the use of vaccination with an avirulent *B. abortus* organism (strain 19) has been observed to cause, in some instances, a loss of appetite. This may result in temporary loss of body weight if it persists. This observation has been noted with calves in the University herd. In addition, during the course of an experiment in which blood levels of vitamin A and ascorbic acid were being followed, vaccination had a material effect on these levels. This led to the present study in which the course of change in blood plasma vitamins as well as temperature, body weight, condition of calves and leucocyte differential were noted.

METHODS

The procedure consisted of recording daily blood plasma levels of vitamin A, ascorbic acid, temperature, body weight and condition of the calves. This was begun 1 day before and continued for 7 days after inoculation. Observations on leucocyte count and differential (Wright stain) were made on the day before and the first, third and fifth days following inoculation. The inoculum consisted of an injection of 5 ml. of a solution containing 10 billion organisms per ml.

Ten heifer calves which were to be vaccinated were placed under observation. These calves, seven Holsteins, two Jerseys and one Guernsey, were divided into four lots as follows: lot I received 0.5 g. ascorbic acid subcutaneously; lot II were the untreated controls; lot III received approximately 6,000 γ vitamin A orally and 0.5 g. vitamin C subcutaneously; and lot IV received a supplement of approximately 6,000 γ vitamin A orally. The supplements in lots I, III and IV were begun the day before inoculation and continued for 7 days thereafter.

The plasma ascorbic acid was determined by the method of Mindlin and Butler (4) and vitamin A by Kimble's method (3).

With the exception of the supplements, treatment consisted of the usual care and feed given calves in the University herd. The pertinent data concerning these calves at the beginning of this study are given in table I.

RESULTS

The results (table 2) indicate that vaccination with *B. abortus* vaccine (strain 19) caused a decrease in blood plasma ascorbic acid averaging 0.18 mg. per 100 ml. in the four lots. This decrease was apparent on the day following inoculation.

Received for publication June 29, 1949.

¹ Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

² Present address: Department of Dairy Husbandry, Purdue University, Lafayette, Ind.

TABLE 1
History of experimental animals

Lot no.	Breed	Age at beginning of exp.	Condition	Previous history regarding Bang's disease
		(d.)		
I				
338	H	215	Normal	Not known
342	H	193	Normal	Not known
II				
334	II	241	Normal	Not known
343	H	157	Normal	Positive reaction in 1:200 dil. at 77 d. ^a
729	J	209	Normal	Not known
III				
340	H	202	Normal	Not known
582	G	237	Normal	Not known
731	J	150	Growth somewhat retarded	Positive reaction in 1:200 dil. at 70 d. ^a
IV				
336	H	231	Normal	Not known
341	H	202	Normal	Not known

^a Had nursed infected cow from birth.

When vitamin C was administered subcutaneously in lots I and III, a drop of the same magnitude occurred, but normal levels of blood plasma ascorbic acid were attained again in less than 7 days. In contrast, the blood plasma ascorbic acid injections, did not reach normal levels within this period.

Blood plasma vitamin A also was affected by this vaccination. The decrease, which amounts on the average to 3.0γ per 100 ml., was greatest on the second day following inoculation rather than on the first, as was the case with ascorbic acid.

TABLE 2
The effect of calfhood vaccination on average blood plasma ascorbic acid, vitamin A and carotene levels, and rectal temperature

Lot no.	Days before and after inoculation							
	- 1	0	1	2	3	4	5	
mg. ascorbic acid/100 ml. blood plasma								
I	0.37	0.49	0.31	0.34	0.32	0.34	0.35	0.40
II	0.50	0.46	0.26	0.24	0.24	0.26	0.27	0.32
III	0.52	0.61	0.40	0.49	0.36	0.48	0.49	0.48
IV	0.38	0.38	0.26	0.23	0.16	0.23	0.23	0.26
γ Vitamin A/100 ml. blood plasma								
I	16		14	12	14	13	14	16
II	15		14	11	12	13	13	15
III	16		18	15	16	18	20	21
IV	18		19	16	17	18	20	21
Rectal temperature (°F.)								
I	102.2	101.8	105.4	105.9	105.1	103.2	101.6	101.8
II	102.1	102.2	105.2	105.2	104.0	102.8	101.7	102.1
III	101.9	101.6	104.7	104.8	103.8	103.0	102.3	102.1
IV	101.8	101.6	105.4	104.6	104.8	103.1	101.8	102.0

TABLE 3
The effect of calfhood vaccination on total and differential leucocyte counts

Lot and number	Days before and after treatment																		
	-1				+1				+3				+5						
	Leucocyte count	Lymphocytes	Neutrophils		Leucocyte count	Lymphocytes	Neutrophils		Leucocyte count	Lymphocytes	Neutrophils		Leucocyte count	Lymphocytes	Neutrophils				
	(mm. ³)	(%)	(%)	(%)	(1 mm. ³)	(%)	(%)	(%)	(1 mm. ³)	(%)	(%)	(%)	(1 mm. ³)	(%)	(%)	(%)			
I																			
338	10,500	77	19	2	2	14,200	34	57	8	1	12,900	27	73	—	10,800	77	5	18	—
342	8,900	65	17	7	11	9,200	61	34	3	2	9,400	71	8	20	1	8,500	79	12	7
Av.	9,700	71	18	5	7	11,700	48	46	6	2	11,150	49	41	10	1	9,650	78	9	13
II																			
334	10,400	68.5	21	9	0.5	12,500	40	50	9	1	13,300	77	21	1	1	11,400	74	15	10
343 ^a	10,200	61	26	6.5	6.5	15,200	30	53	14	3	12,300	64	21	14	1	8,200	73	11	17
729	7,700	81	15	3	1	10,000	33	60	5	2	7,900	65	24	11	—	7,200	77	7	15
Av.	9,433	70.5	21	6.2	2.7	12,566	34	54	9	2	11,170	69	22	9	1	8,933	75	11	14
III																			
340	8,300	67	17	15	1	9,100	47	52	1	—	7,100	63	22	14	1	6,300	76	10	13
582	10,100	82	11	6.7	0.3	9,200	42	47	10	1	7,500	69	13	18	—	8,700	67	11	21
731 ^a	6,900	71	12	15	2	13,400	42	54	23	1	11,000	68	23	8	1	8,700	70	14	15
Av.	8,433	73	13	12.2	1.1	10,566	44	51	11	1	8,533	67	19	13	1	7,900	71	12	16
IV																			
336	9,800	58	26	14	2	11,700	44	45	10	1	10,800	72	21	5	2	8,500	83	10	7
341	6,900	70	15	13	2	10,700	42	50	7	1	9,200	70	19	11	—	7,700	64	19	16
Av.	8,350	64	21	14	2	11,200	43	48	9	1	10,000	71	20	8	1	8,100	74	15	12

^a These calves were known to have had a positive reaction in 1:200 dil. at 77 and 70 d. respectively.

Calves which received vitamin A supplements responded in the same way as those which received no supplement, except that blood concentrations were maintained at a higher level.

The calves showed no significant change in weight during the course of this study. This was in contrast to that noted in the preliminary observation mentioned above. Inappetence was observed only on the day following inoculation, with a complete return to normal on the third day.

The rectal temperature showed an average rise of 3.4° F. on the first day following inoculation. This higher temperature was apparent for 2 days, after which it receded, reaching normal levels by the fifth day. There was no difference in the course of temperature rise among the calves of the various lots.

The leucocyte count showed a distinct rise on the first day following inoculation in all but one calf and returned to normal by the fifth day (table 3). The leucocyte count of calves 338, 334, 343 and 731 reached somewhat higher levels than for the remaining six calves. Two of these, 343 and 731, were known to have had a positive reaction to *B. abortus* previously. No such information was available on the remaining calves.

The lymphocytes decreased from an average of 70.05 per cent to 41.50 per cent and neutrophils increased from an average of 17.90 per cent to 50.2 per cent on the day following inoculation. These, likewise, had returned to near normal levels by the fifth day. The monocytes and eosinophiles were affected only slightly.

DISCUSSION

The results of this study indicate that vaccination with *B. abortus* vaccine (strain 19) has a depressing effect on blood plasma levels of vitamin A and C. The ascorbic acid does not reach normal levels during the first week following vaccination. Since this vaccination generally is practiced when calves are 5 to 8 months of age, and normal blood plasma vitamin C levels are in the neighborhood of 0.4 to 0.6 mg. per 100 ml., it seems logical to assume that no detrimental effect will result from the temporary lower levels, which in these calves range from 0.16 to 0.24 mg. per 100 ml. This might not be true if calves were deficient at the time of inoculation. The drop in blood plasma vitamin A observed in these calves was consistent but not marked, and the data do not indicate that deficient levels were reached. Supplementation of the normal diet with either ascorbic acid or vitamin A did not alter the course of blood plasma levels, except to maintain higher concentrations.

The rise in rectal temperature follows closely the drop which occurred in blood plasma ascorbic acid. A drop in the concentration of blood plasma ascorbic acid apparently correlates closely with the development of an increased body temperature.

The leucocyte count is of interest since two of the four calves which reached counts above the rest were known to have had a previous positive reaction. The question is raised as to whether the two calves with higher leucocyte counts, but on which no titre had been determined previously, had a positive reaction at some

earlier date. These data, although from limited numbers, definitely demonstrate a rise in leucocyte count immediately following the vaccination. This average is transitory, since levels approximating normal values are reached in a matter of a few days. The reports by Bell and Irwin (1) and Irwin and Bell (2) do not indicate a rise in adult animals during an infection with *B. abortus*. However, their data are averages based on longer time intervals, and do not emphasize the fluctuations in leucocytes immediately following the infection.

SUMMARY

These studies show that blood plasma vitamin C and A levels are reduced by vaccination with *B. abortus* vaccine (strain 19). The vitamin C levels do not return completely to normal after 1 wk. as do the vitamin A levels. Vitamin C or A in addition to the normal diet had no effect on the course of blood plasma levels, except to maintain them above that for calves not receiving the supplement.

A rise in rectal temperatures followed closely the drop in blood plasma vitamin C and may suggest a relationship between the increase in body temperature and a lowering of blood plasma ascorbic acid.

Leucocyte count increased the day following inoculation. Differential count showed that the neutrophiles increased, the lymphocytes decreased, and the monocytes and eosinophiles slightly increased and decreased, respectively, by this type of vaccination.

ACKNOWLEDGMENT

The authors are grateful for the cooperation of J. H. Shaw, now with the Harvard Dental School, and of C. K. Whitehair, now with the Animal Husbandry Department, Oklahoma A. and M. College.

REFERENCES

- (1) BELL, F. N., AND IRWIN, M. R. Studies on the Variation of the Blood Cells of Cattle in Health and during *Brucella* Infections. *J. Infectious Diseases*, **63**: 251. 1938.
- (2) IRWIN, M. R., AND BELL, F. N. The Interrelationships of the Blood Cells of Cattle in Health and during *Brucella* Infections. *J. Infectious Diseases*, **63**: 263. 1938.
- (3) KIMBLE, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, **24**: 1055. 1939.
- (4) MINDLIN, R. L., AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma; A Macromethod and Micromethod. *J. Biol. Chem.*, **122**: 673. 1938.

PREPARTUM MILKING. II. THE EFFECT OF PREPARTUM MILKING ON THE CAROTENE AND VITAMIN A AND PROXIMATE COMPOSITION OF COLOSTRUM¹

H. D. EATON, R. E. JOHNSON, A. A. SPIELMAN, L. D. MATTERSON AND L. NEZVESKY
Animal Industries Department, Storrs Agricultural Experiment Station, Storrs, Connecticut

Prepartum milking for 10 days prior to parturition has been found by several investigators (4, 7, 11, 12, 13) to result in the production of colostrum which resembles milk in proximate composition. In addition, the proportion of various proteins, albumin, casein and globulin are altered markedly. The carotene content of colostrum from cows milked prepartum has been found (6) to rise on the day of parturition. No data were found in the literature as to the effect of prepartum milking on the vitamin A content of colostrum.

The objectives of this study were (a) to determine the effect of prepartum milking for 10 days prior to the calculated parturition date on the carotene, vitamin A and proximate composition of colostrum, and (b) to follow the changes occurring in the carotene and vitamin A content of the pre-milk and colostrum of cows milked prepartum. Secondly, these factors were studied in relation to two dietary regimes.

EXPERIMENTAL

Animals. A total of 44 cows of the Ayrshire, Guernsey, Holstein and Jersey breeds in the University of Connecticut herd which calved from November, 1947, through December, 1948, were used in this experiment. The treatment of these cows and changes in various blood constituents have been described in the first paper of this series (3). Briefly, they represented four experimental groups: 1-A, postpartum milked—basal ration; 1-B, postpartum milked—basal ration plus 1 million USP units of vitamin A daily for 30 days prior to the calculated parturition date; 2-A, prepartum milked for 10 days prior to calculated parturition date—basal ration; and 2-B, prepartum milked—basal ration plus vitamin A. Each experimental group in this study represented a total of 11 cows.

Samples and Analyses. All prepartum milkings and the first six postpartum milkings were sampled. The samples were chilled immediately and held at 4° C. in the dark until analyzed, in most cases within 6 days after collection. Aliquot samples from daily prepartum milkings and from each milking postpartum were analyzed for carotene, vitamin A, protein, lactose, fat and ash. In addition, specific gravity was determined. The methods were the same as those reported previously (2). In cases where the cows milked prepartum calved between the morning and evening milkings, the morning sample was analyzed separately.

Standard statistical procedures (9), such as analysis of variance, were used to test for differences between treatments.

Received for publication July 7, 1949.

¹ This work was supported in part by the Big-Y-Foundation, Norwich, Conn. and Chas. M. Cox Co., Boston, Mass.

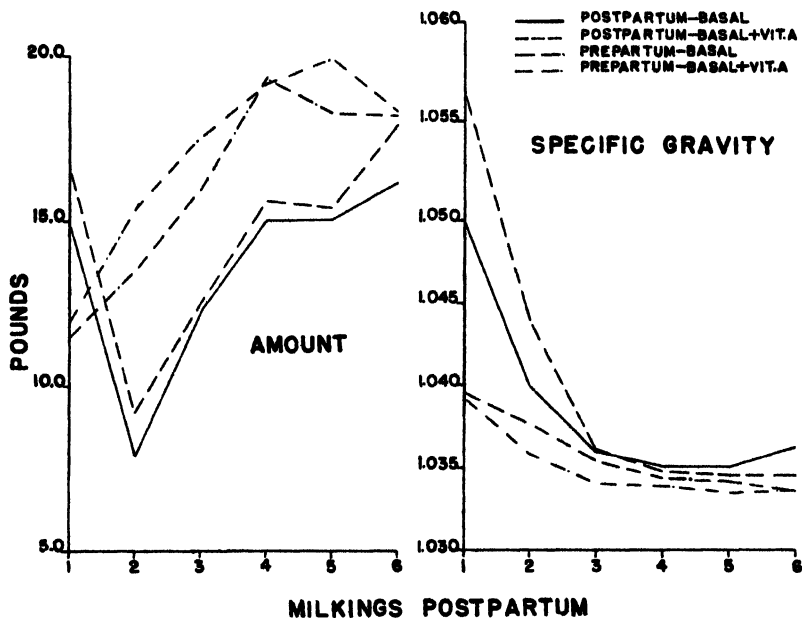


FIG. 1. The effect of prepartum milking on the amount and specific gravity of colostrum.

RESULTS

Data on the pounds of colostrum, the specific gravity, and the carotene, vitamin A and proximate composition for the first six milkings postpartum of all

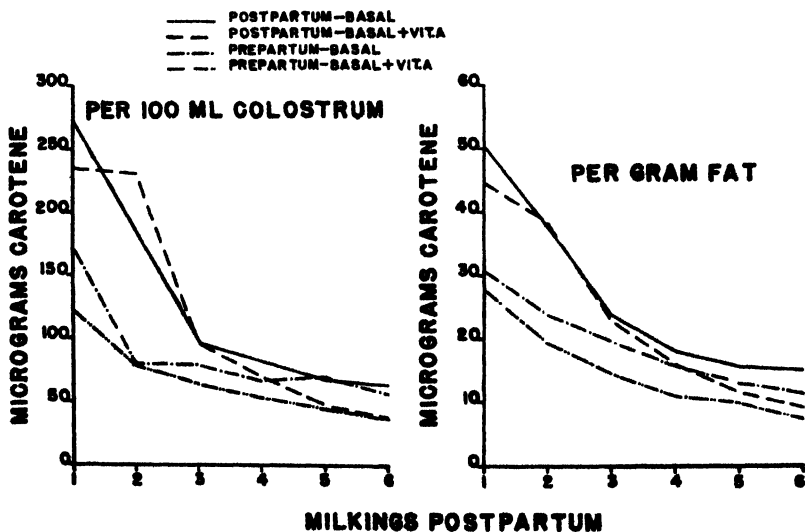


FIG. 2. The effect of prepartum milking on the carotene content of colostrum.

the experimental animals are given in figures 1, 2, 3 and 4. Mean values for the carotene and vitamin A contents of the pre-milk and colostrum for the 14 cows milked 10 or more days prepartum are represented in figures 5 and 6. In general, prepartum milking resulted in colostrum which was lower in carotene, vitamin A, protein and ash and higher in lactose than colostrum from cows milked only postpartum. In those cows milked prepartum for 10 days or longer and fed only the basal ration, there was a negative trend in the carotene and vitamin A contents of both pre-milk and colostrum. Parturition appeared to arrest temporarily this negative trend. Supplementary prepartum feeding of vitamin A raised the level of vitamin A in both the pre-milk and the colostrum and lowered the level of carotene in the colostrum.

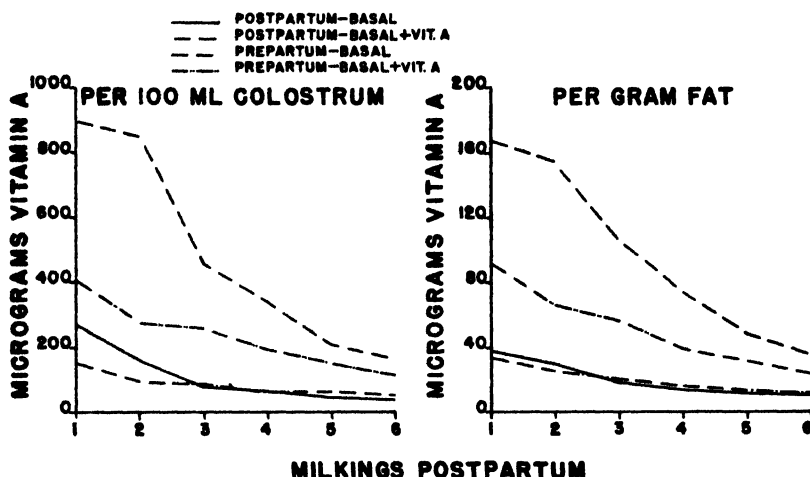


FIG. 3. The effect of prepartum milking on the vitamin A content of colostrum.

With the exception of the first milking postpartum, the average amount of colostrum (fig. 1) from the next five milkings was significantly less in the cows milked only postpartum ($P < 0.05$). It was observed at the second milking of many of the cows milked only postpartum that there was incomplete "let-down" of milk. The specific gravity of the colostrum averaged less ($P < 0.001$) in the first six milkings postpartum of those cows which had been milked prepartum than in those that had not.

The carotene content (fig. 2) per 100 ml. of colostrum or per gram of colostrum fat averaged less ($P < 0.05$ on a volumetric basis and $P < 0.10$ on a per gram of fat basis) in the cows milked prepartum. The average carotene levels were less following the prepartum feeding of vitamin A supplements, but the difference was not significant. The vitamin A content, expressed as micrograms per 100 ml. of colostrum or as micrograms per gram of colostrum fat (fig. 3) also averaged less ($P < 0.01$) in the cows milked prepartum. The prepartum feeding of vitamin A supplements increased the average level of vitamin A in the

colostrum very significantly ($P < 0.001$). This increase was large enough to more than offset the usual reduction in the vitamin A content of the colostrum due to prepartum milking ($P < 0.01$).

The average protein and ash contents of the colostrum were lower ($P < 0.001$ for the protein and $P < 0.01$ for the ash) and the average lactose content higher

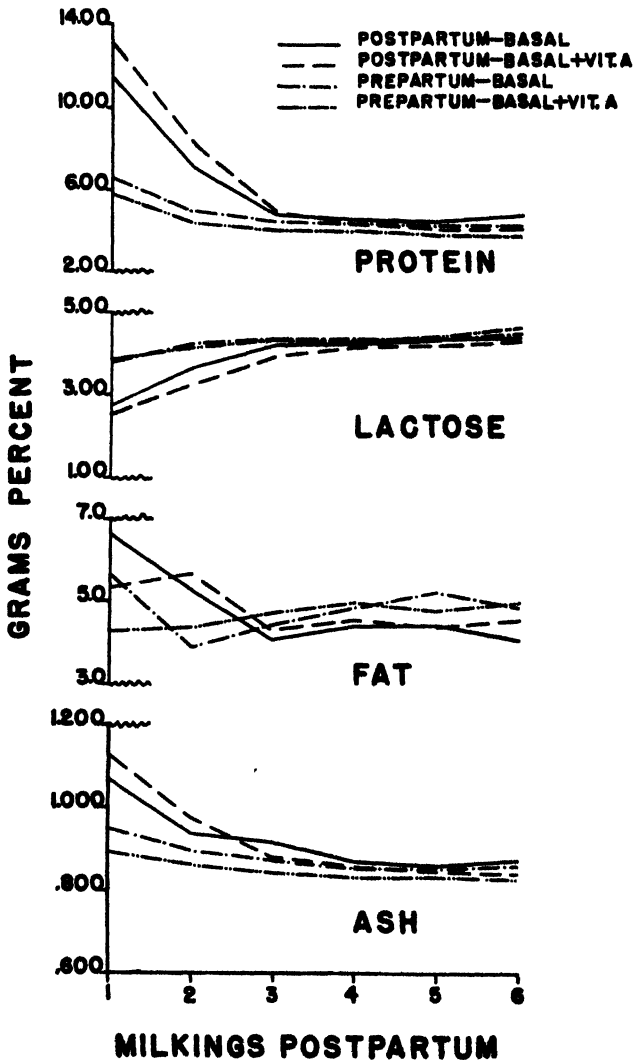


FIG. 4. The effect of prepartum milking on the proximate constituents of colostrum.

($P < 0.001$) (fig. 4) as a result of prepartum milking, but the average fat content was not affected significantly. The feeding of supplementary vitamin A did not influence the proximate constituents.

The carotene and vitamin A content of both pre-milk and colostrum (fig. 5 and 6) decreased with successive milkings.² The decrease in animals receiving only the basal ration was statistically significant when the carotene and vitamin A contents were expressed in units per gram of colostral fat ($P < 0.01$). The greater variability among the cows receiving the supplementary vitamin A reduced the statistical significance of the change considerably, even when vitamin A content was expressed in terms of the colostral fat ($P < 0.05$). Just prior to parturition

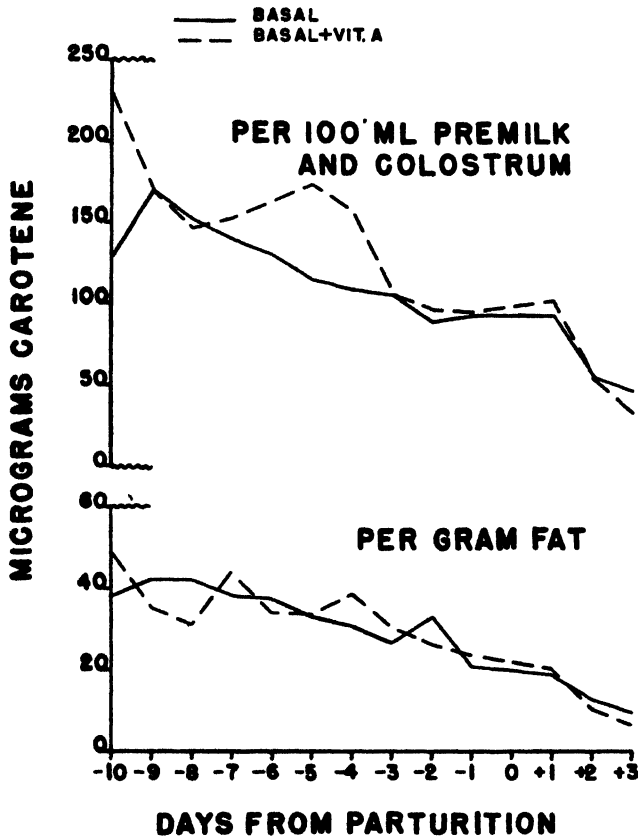


FIG. 5. The effect of prepartum milking on the trends in the carotene content of both pre-milk and colostrum.

there was little change in either the carotene or vitamin A content of the pre-milk, so that from the third day prior through the first day following parturition there were no significant differences between days. Therefore, it is reasonable to assume, that parturition does arrest temporarily the drop in both the carotene and vitamin A contents of the pre-milk and colostrum. The prepartum feeding of

² Similar data were obtained on the proximate principles which agreed with those in the literature.

supplementary vitamin A resulted in higher average levels ($P < 0.05$) of vitamin A in the pre-milk and colostrum, but had no noticeable effect on the carotene content.

DISCUSSION

These data indicate that both management and diet may influence markedly the composition of colostrum. Prepartum milking decreases both the carotene and the vitamin A content of colostrum. However, the prepartum feeding of

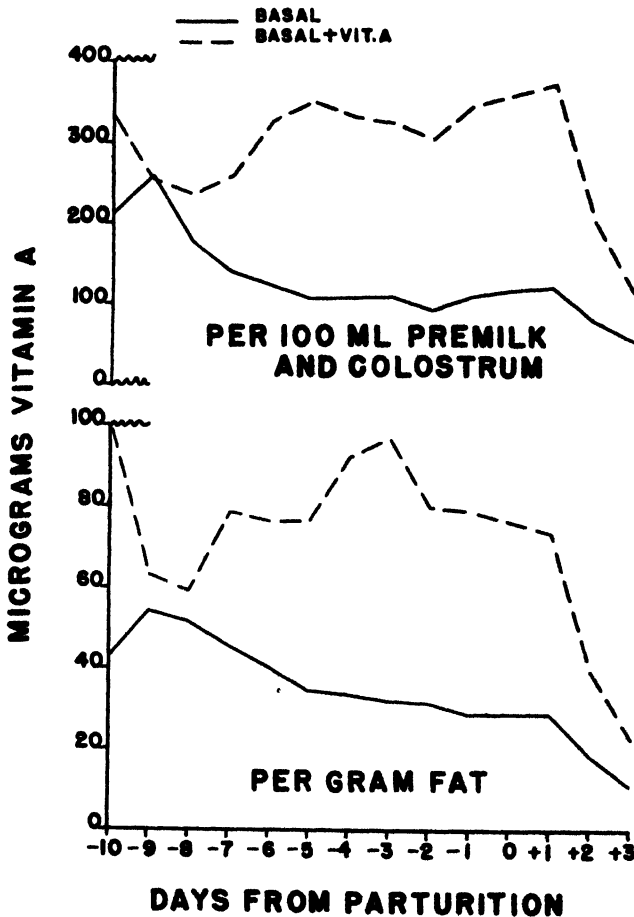


FIG. 6. The effect of prepartum milking on the trends in the vitamin A content of both pre-milk and colostrum.

supplementary vitamin A can maintain higher levels of vitamin A in cows milked prepartum than are found in cows milked only postpartum and fed only on the basal ration. The effect of prepartum feeding of vitamin A supplements on the carotene and vitamin A contents of colostrum in the cows milked only postpartum agrees with the recent data reported by Parrish *et al.* (8) and others (5, 10).

The changes in the proximate constituents of colostrum from cows milked prepartum also are in agreement with previous studies. Prepartum milking for a week or more before parturition has been shown to result in colostrum closely resembling normal milk in proximate composition (7, 11) and to decrease the globulin and albumin components of the protein (7, 11, 12, 13). Similar results have been reported for continuous milking from one lactation to the next (4) and for the initiation of lactation before pregnancy or early in pregnancy (1, 14).

The decrease in carotene and vitamin A with successive prepartum milkings follows the trends reported for the protein, ash, and lactose (7, 11, 12, 13). Although there was a tendency for both carotene and vitamin A to be affected by parturition in the study reported herein, the magnitude of this change and the trends in the carotene levels of pre-milk and colostrum are not in agreement with those reported by Keyes *et al.* (6). When carotene and vitamin A are related to the content of fat, the changes occurring at parturition are even less apparent. It is of interest that Eckles and Palmer (4) also reported an increase in the albumin and globulin at the time of calving in cows milked continuously from the previous lactation, although later workers (7, 11, 12, 13) did not confirm these findings.

SUMMARY

The effect of prepartum milking for 10 days prior to the calculated parturition date on the carotene, vitamin A and proximate composition of colostrum has been studied in 44 cows. In addition, the changes occurring in the carotene and vitamin A contents of both pre-milk and colostrum were observed in 14 of these cows milked for 10 or more days prepartum. Secondarily, the effect of feeding one million USP units of vitamin A daily for 30 days prior to the calculated parturition date was measured.

Prepartum milking resulted in significant decreases in the carotene, vitamin A, protein and ash contents and in the specific gravity and significant increases in the lactose content of the colostrum from the first six milkings postpartum. Both carotene and vitamin A decreased with successive milkings in the pre-milk and colostrum of cows milked for more than 10 days prepartum. Parturition affected this decrease by temporarily causing a decrease in the negative trend.

The prepartum feeding of supplementary vitamin A increased significantly the vitamin A content of both pre-milk and colostrum and decreased the carotene content of colostrum.

ACKNOWLEDGMENTS

The authors are most grateful to F. Warren and G. Farrington for the care of the experimental animals and to J. Satchell and R. J. Slate, and to Misses R. J. Caverno, M. W. Dicks and J. H. Kramer for technical assistance at various times during the experiment. Further acknowledgment is due C. I. Bliss, Storrs Agricultural Experiment Station Biometrician, for considerable aid in the statistical analyses of the data and in preparation of the paper.

REFERENCES

- (1) ASDELL, S. A. The Inception of Lactation in the Cow and Goat. *J. Agr. Sci.*, 15: 358-374. 1925.
- (2) EATON, H. D., JOHNSON, R. E., MATTERSON, L. D., AND SPIELMAN, A. A. The Carotene and Vitamin A and Proximate Composition of Portions of the First Milking Postpartum. *J. Dairy Sci.*, 32: 587-595. 1949.
- (3) EATON, H. D., JOHNSON, R. E., HELMBOLDT, C. F., SPIELMAN, A. A., MATTERSON, L. D., JUNGHEER, E. L., KRAMER, J. H., AND SLATE, R. J. Prepartum Milking. I. The Effect of Prepartum Milking on Some Blood Constituents of the Cow. *J. Dairy Sci.*, (submitted for publication). 1949.
- (4) ECKLES, C. H., AND PALMER, L. S. The Influence of Parturition on the Composition and Properties of the Milk and Milk Fat of the Cow. *J. Biol. Chem.*, 27: 313-326. 1916.
- (5) ESH, G. C., SUTTON, T. S., HIBBS, J. W., AND KRAUSS, W. E. The Effects of Soyaphosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, 31: 461-478. 1948.
- (6) KEYES, E. A., REID, J. J., BECHDEL, S. I., BORLAND, A. A., BEAM, A. L., AND WILLIAMS, P. S. Pre-partum Milking (Abstract). *J. Dairy Sci.*, 27: 638-639. 1944.
- (7) MUKHERJEE, S. K., SWAMINATHAN, K., AND VISWANTH, B. The Composition of Pre-milk and Colostrum of Dairy Cows of the Pedigree Sahiwal Herd at the Imperial Agricultural Institute, New Delhi. *Indian J. Vet. Sci.*, 14: 232-239. 1944.
- (8) PARRISH, D. B., WISE, G. H., ATKESON, F. W., AND HUGHES, J. S. Properties of the Colostrum of the Dairy Cow. III. Several Factors Affecting Vitamin A and Carotenoid Content. *J. Dairy Sci.*, 32: 209-221. 1949.
- (9) SNEDECOR, G. W. *Statistical Methods*. 4th ed. Iowa State College Press, Ames. 1946.
- (10) SPIELMAN, A. A., THOMAS, J. W., LOOSLI, J. K., WHITING, F., NORTON, C. L., AND TURK, K. L. The Relationship of the Prepartum Diet to the Carotene and Vitamin A Content of Bovine Colostrum. *J. Dairy Sci.*, 30: 343-350. 1947.
- (11) TURNER, C. W. The Development of the Mammary Gland as Indicated by the Initiation and Increase in the Yield of Secretion. *Mo. Agr. Expt. Sta. Research Bull.* 156. 1931.
- (12) VAN LANDINGHAM, A. H., WEAKLEY, C. E., JR., ACKERMAN, R. A., AND HYATT, G., JR. The Effect of Preparturient Milking on the Composition of Colostrum (Abstract). *J. Dairy Sci.*, 31: 685. 1948.
- (13) VAN LANDINGHAM, A. H., WEAKLEY, C. E., JR., ACKERMAN, R. A., AND HYATT, G., JR. The Relationship of Production of Heifers Milked Prepartum to the Composition of Colostrum. *J. Dairy Sci.*, 32: 559-564. 1949.
- (14) WOODMAN, H. E., AND HAMMOND, J. The Composition of Secretions Obtained from the Udders of Heifers During Pregnancy. *J. Agr. Sci.*, 13: 180-191. 1923.

PARTURIENT PARESIS. III. A STUDY OF VARIOUS BLOOD CONSTITUENTS AT PARTURITION IN MASTECTOMIZED COWS^{1, 2}

R. P. NIEDERMEIER, VEARL R. SMITH AND C. K. WHITEHAIR³

Departments of Dairy Husbandry and Veterinary Science, University of Wisconsin

Mastectomized animals have been used to study various phenomena related to lactation and reproduction. Bert (5), Moore and Parker (25), Marshall and Kirkness (24) and Porcher (28) analyzed the blood and urine of mastectomized animals before and after parturition to determine the site of lactose formation. Shattock (33) removed the mammary glands from guinea pigs to ascertain whether or not the mammary gland produced an internal secretion necessary for growth of the fetus. Sellheim (31) postulated that eclampsia was caused by toxins formed in the mammary gland and reported recovery from eclampsia after removal of the breasts of one patient. A review of some of the early European literature on the relation of the udder to reproduction is covered by Addis (1). Eddington (9) used udderless cows to study the role of the udder in dissemination of *Brucella abortus*. Analyses for blood calcium and phosphorus near the time of parturition were made by Wilson and Hart (37) on a mastectomized goat.

Many workers have proven conclusively that at the time of parturient paresis there is a drop in total serum calcium as well as inorganic phosphorus levels in the blood, usually accompanied by an increase in the magnesium level (2, 4, 8, 10, 11, 12, 17, 18, 20, 21, 22, 23, 28, 29, 30, 34, 35). These changes seem to be accentuations of the trends in the blood mineral picture in normal parturitions (2, 8, 16, 17, 27, 37). The decrease in serum calcium and plasma phosphorus may be caused by either an increased demand for serum calcium and phosphorus as the result of the initiation of lactation, or physiological changes characteristic of parturition which may influence the blood mineral picture or a combination of the two. If the sudden demand for calcium and phosphorus at the onset of lactation is solely responsible, then initiation of lactation before parturition should reduce the incidence of parturient paresis. However, studies reported by Smith and Blosser (36) showed that prepartum milking had little or no effect on reducing the incidence of parturient paresis. Also, Niedermeier and Smith (26) observed parturient paresis in a Jersey cow not milked following parturition.

Received for publication July 9, 1949.

¹ Part of these data were taken from a thesis presented by R. P. Niedermeier to the graduate faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

² Published with approval of the director of the Wisconsin Agricultural Experiment Station.

³ Present address: Animal Husbandry Department, Oklahoma A & M College, Stillwater.

This study was undertaken to ascertain the effect of parturition uncomplicated by the initiation of lactation on some of the blood constituents at calving, with emphasis upon those constituents particularly related to the milk fever syndrome.

EXPERIMENTAL

Four Jersey cows and one Guernsey cow were mastectomized according to the technique described by Frank (15). Epidural and local anaesthesia were used, and each half of the udder was removed separately. The principal blood vessels were ligated. The cut edges of the skin were sutured together and pressure body bandages were applied to control postoperative bleeding. Physiological saline was given after the operation as prophylactic treatment to prevent shock. Recovery was rapid with no apparent abnormal effects on the animals.

TABLE 1
History of cows used in study

Cow no.	Age (yr.)	No. of parturition	Previous at- tacks of par- turient paresis	Breed	Date of parturition
Experimental					
AS-19	10	8	2	Jersey	4- 9-47
1-J	2	1	0	Jersey	1-13-47
1-J	3	2	0	Jersey	1-28-48
1-J	4	3	0	Jersey	1- 8-49
731	5	3	0	Jersey	1-11-48
695	8	5	1	Jersey	4- 9-48
OY-23	†	†	3	Guernsey	5-20-48
OY-23	†	†	3	Guernsey	4-30-49
Control					
720	7	4	0	Jersey	1- 5-49
732	5	3	0	Jersey	1-17-48
696	8	5	0	Jersey	4-12-48
589	4	2	0	Guernsey	5-24-48
559	7	5	0	Guernsey	4-24-49

Five intact cows were paired with some of the mastectomized cows to serve as controls. The controls were selected to correspond with the mastectomized cows in respect to age, number of parturitions and date of parturition, and were subjected to the same feeding and management procedures. Cow 720 was the control for the third parturition of 1-J; 732 for 731; 696 for 695; 589 for the first parturition of OY-23; and 559 for the second parturition of OY-23. Calving dates and histories of these animals are presented in table 1. Blood samples were taken at approximately the same time daily, usually beginning the fifth day before the expected calving date and continued for 5 days postpartum. On the day of calving, samples were taken at 1 to 3, 5 to 7, and 9 to 11 hours after parturition.

Serum calcium was determined by the Clark and Collip (6) method, plasma phosphorus by the Fiske and Subbarow (14) method, serum magnesium by the method of Simonsen *et al.* (32), and plasma fat by Allen's (3) method.

RESULTS

The results of analyses of the various blood constituents for mastectomized and control cows are presented in tables 2, 3, 4 and 5. Averages given include only the five mastectomized parturitions for which there were control cows.

The serum calcium levels of the cows are presented in table 2. These results show that the serum calcium level of the mastectomized cows decreased slightly on the day of calving, with a very rapid recovery and an increase in postpartum levels above the prepartum level. This is in contrast to the charac-

TABLE 2

Comparison of the serum calcium before and after parturition of mastectomized and intact cows

Cow	Blood serum calcium (mg. %)												
	Days prepartum					Day of parturition			Days postpartum				
	5	4	3	2	1	1-3 hr.	5-7 hr.	9-11 hr.	1	2	3	4	5
Experimental													
AS-19	10.6	11.2	11.0	10.8	10.8	9.4	10.1	10.6	11.5	11.7	11.3	11.0	10.3
1-J	10.9	11.2	11.5	11.4	11.2			11.2	10.8	11.0	10.5	11.0	11.4
1-J	10.2		10.3	10.6	11.2	10.6	10.6	10.6	10.4	10.9	10.8	11.1	10.8
1-J	11.3	10.3	9.8	11.3	10.8	10.0	9.6	10.7	10.7	10.8	11.2	10.9	10.7
731	10.4	10.5	10.2	10.2	10.2	9.9	9.9	10.2	11.0	10.7	10.8	10.6	10.1
695	9.6	9.7	9.5	9.5	9.7		9.4	9.3	10.2	10.4	10.5	10.6	10.7
OY-23	10.4	10.1	10.5	10.3	10.2	9.9	10.3	10.8	10.8	11.6	11.3	11.1	11.3
OY-23	10.0	10.8	9.6	10.5	10.6	10.9	10.3	10.7	10.9	11.0	11.0	10.8	10.9
Control													
720			10.2	10.9	10.0	9.2	9.6	9.6	9.3	9.1	9.5	9.8	10.1
732	11.0	11.4	11.1	10.8	9.8	8.8	8.5	8.3	7.8	9.8	11.3	11.0	10.3
696	10.3	10.5	10.5	9.4	10.4	6.5	5.8	5.7	6.5	6.2	6.9	6.9	
589			11.1	11.2	10.7	10.5	9.5	10.2	9.3	10.4	10.7	10.5	10.4
559	11.2	11.6	11.6	11.6	11.3	8.9	10.0	9.3	9.5	9.2	9.9	10.2	9.9
Average of five experimental parturitions and their controls													
Experi- mental			9.9	10.4	10.3	10.2	9.9	10.3	10.7	10.9	11.0		
Control			10.9	10.8	10.4	8.8	8.7	8.6	8.5	8.9	9.7		

teristic change shown in the control cows. The serum calcium of the control cows declined from a level of 10.4 mg. per cent 1 day prepartum to a low of 8.5 mg. per cent 1 day postpartum, and the subsequent recovery period was much slower than that of the experimental cows. This perceptible drop in the serum calcium level occurred in each of the control cows, and with cow 696 the level was close to that found in milk fever. In the mastectomized cows an examination of the individual cow data reveals no such consistent trend, and where the drop did occur it was of much less magnitude.

Plasma phosphorus levels of the two groups, as shown in table 3, in general paralleled each other, with a slightly higher level throughout the sampling period for the mastectomized animals. As in the case of calcium, the control

TABLE 3

Comparison of the inorganic plasma phosphorus before and after parturition of mastectomized and intact cows

Cow	Blood plasma inorganic phosphorus (mg. %)												
	Days prepartum					Day of parturition			Days postpartum				
	5	4	3	2	1	1-3 hr.	5-7 hr.	9-11 hr.	1	2	3	4	5
Experimental													
1-J	4.6	4.5	5.5	5.0	4.2	2.7	3.8	4.6	4.9	5.2	5.2	4.6	4.3
1-J	5.1	4.8	5.1	5.1	4.2	3.1	3.9	4.6	4.5	6.9	6.4	6.5	6.1
731	4.2	4.8	3.9	4.8	3.7	3.2	4.8	4.4	3.9	4.1	5.1	4.9	
695	6.4	4.7	5.0	5.7	4.8		4.8	3.2	3.6	3.2	3.7	3.9	4.1
OY-23	3.7	3.6	3.6	3.9	3.8	2.8	3.7	3.0	2.4	3.3	3.9	3.9	4.0
OY-23	4.3	7.7	6.7	6.4	4.7	5.3	4.3	4.3	3.9	7.2	6.9	6.8	7.2
Control													
720			3.7	3.6	3.2	2.7	2.9	2.6	2.7	3.4	4.0	4.7	4.8
732	3.2	3.8	3.9	4.1	2.4	2.3	3.2	3.2	3.5	4.1	4.8	3.9	5.6
696	4.3	4.4	5.2	5.3	5.0	1.0	1.3	2.7	3.1	4.6	6.0	4.4	4.0
589			2.8	3.8	2.4	2.4	2.7	2.6	4.2	4.2	3.7	3.9	3.0
559	5.2	6.1	5.6	6.6	4.0	2.8	2.5	3.1	3.9	4.2	4.6	5.3	5.4
Average of five experimental parturitions and their controls													
Experi- mental			4.9	5.2	4.2	2.9	4.3	3.9	3.7	4.9	5.2		
Control			4.2	4.7	3.4	2.2	2.5	2.8	3.5	4.1	4.6		

TABLE 4

Comparison of the serum magnesium before and after parturition of mastectomized and intact cows

Blood serum magnesium (mg. %)													
Cow	Days prepartum					Day of parturition			Days postpartum				
	5	4	3	2	1	1-3 hr.	5-7 hr.	9-11 hr.	1	2	3	4	5
Experimental													
1-J	2.5		2.6	2.3	2.7	2.4	2.6	2.5	2.4	2.7	2.5	2.4	2.6
1-J	2.7	2.9	2.5	3.3	2.7	3.0	2.8	2.8	2.4	2.3	2.6	2.4	2.4
731	2.1	2.4	2.3	2.4	2.4	2.5	2.5	2.5	2.4	2.4	2.3	2.1	2.4
695	3.0	2.8	2.6	2.4	2.4		2.4	2.4	1.9	1.9	2.0	2.2	2.4
OY-23	2.1	2.4	2.4	2.5	2.7	2.5	2.5	2.3	2.4	2.4	2.4	2.4	2.4
OY-23	2.7	2.8	2.6	2.6	2.6	2.6	2.7	2.7	2.5	2.4	2.4	2.6	2.6
Control													
720			2.6		2.8	2.9	3.3	3.1	3.2	2.9	2.7		2.2
732	2.4	2.6	2.6	2.7	2.7	3.3	3.4	3.7	3.4	2.9	2.1	2.3	2.3
696	2.3	2.4	2.5	2.5	2.6	4.2	4.3	4.1	4.2	4.0	2.8	1.9	1.8
589			2.7	2.7	2.7	2.7	2.5	2.4	2.5	2.5	2.4	2.2	2.0
559	2.5	2.5	2.1	2.2	2.5	3.0	3.2	2.8	3.2	2.7	2.2	2.1	2.0
Average of five experimental parturitions and their controls													
Experi- mental			2.5	2.6	2.6	2.6	2.6	2.5	2.3	2.3	2.3		
Control			2.5	2.5	2.7	3.2	3.3	3.2	3.3	3.0	2.4		

group shows the more consistent trend, inasmuch as the experimental group has a definite increase in the 5 to 7 hr. period as compared to the 1 to 3 and 9 to 11 hr. periods. This very possibly would disappear if larger numbers were involved. Table 4 points out a strikingly different serum magnesium level between the experimental and control animals. The serum magnesium level of the mastectomized cows remained quite constant, with a slightly lower level in the postpartum period beginning on the day of calving. In the control cows the average began to increase the two days prepartum, beginning at 2.5 mg. per cent and increasing to 3.3 mg. per cent 1 to 3 hr. postpartum. This was followed

TABLE 5

Comparison of the plasma fat before and after parturition of mastectomized and intact cows

Blood plasma fat (mg. %)													
Cow	Days prepartum					Day of parturition			Days postpartum				
	5	4	3	2	1	1-3 hr.	5-7 hr.	9-11 hr.	1	2	3	4	5
Experimental													
AS-19	170	213	214	225	158	161	158	157	199	205	192	184	177
1-J	166	174	211	192	194	197	187	192	202	205	226	205	210
1-J	216	202	215	211	196	201	179	194	187	175	196	187	191
731	149	154	137	133	145	124	127	129	141	133	132	155	145
695	186	173	162	165	157		161	156	153	152	156	164	161
OY-23	216	216	214	219	207	187	240	221	241	219	225	236	246
OY-23	203	199	202	188	210	199	180	178	177	190	185	175	185
Control													
720			209	186	172	145	158	164	165	162	174	164	164
732	144	164	154	149	144	123	110	120	116	119	109	162	153
696		193	189	165	178	131	128	127	103	109	130	122	136
589			206	198	185	183	172	168	168	154	149	149	174
559	209	206	201	192	180	153	156	155	158	167	149	144	132
Average of five experimental parturitions and their controls													
Experi- mental			186	183	183	178	177	176	180	174	179		
Control			192	178	172	147	145	147	142	142	142		

by a gradual decline to prepartum levels by the third day postpartum. Control cow 589 had no increase in her serum magnesium level.

There was little change in the plasma fat levels of the mastectomized cows as a result of parturition. The control cows showed a rather constant decline in the plasma fat from the third day prepartum to 1 to 3 hr. postpartum, with the level remaining quite constant from 1 to 3 hr. to 3 days postpartum.

DISCUSSION

As indicated by the foregoing data and results, the blood mineral levels of calcium and magnesium are markedly different for the two groups. The control cows exhibit the characteristic decrease in serum calcium at parturition reported

by many workers in other than first calf heifers. The mastectomized cows exhibit little change as compared to the cows calving with intact udders. An increase in blood magnesium levels in cows with parturient paresis has been reported by numerous workers (2, 4, 17, 29, 30, 35). They also have shown a tendency in this direction in normal calvings. It was suggested by Hibbs *et al.* (20) that the rise in magnesium may be explained as a compensatory mechanism for the concurrent drop in calcium and phosphorus. Using this line of reasoning, one might assume that the increase in magnesium did not occur in the mastectomized cows because a very small drop in the serum calcium level occurred. A major portion of the difference in the magnitude of the drop in serum calcium between the two groups logically may be attributed to the mobilization of calcium for the secretion of milk in the cows calving with intact udders.

In contrast to the other minerals studied, the plasma phosphorus levels for the two groups were similar in their magnitude of decrease. Palmer *et al.* (27) noted an appreciable increase of inorganic phosphorus 15 min. after exercise, followed by a marked decrease which persisted for at least 2 hr. after exercise. The exercise involved in parturition may contribute to the drop in plasma phosphorus. The decrease of the plasma phosphorus in both groups is of the magnitude of that reported by other workers for normal calvings of cows with intact udders. No significance is attached to the slight differences in prepartum levels of calcium or phosphorus between the experimental and control groups, inasmuch as they fall within the normal range reported by Alleroft (2).

The decrease in the plasma fat levels of the normal cows at parturition is in agreement with the results reported by Allen (3). Compared with the control group, the mastectomized cows showed little change in plasma fat levels.

SUMMARY

The serum calcium and magnesium, and the plasma phosphorus and fat levels have been determined for 5 days prepartum, three times on the day of parturition, and 5 days postpartum for eight parturitions of five mastectomized and five parturitions of five intact cows.

Data are presented showing a greater, more consistent decline in the serum calcium and plasma fat of the intact cows than the mastectomized cows. Serum magnesium levels increased in the cows calving with intact udders and showed no appreciable change in the mastectomized cows. Plasma phosphorus levels showed a drop in both groups of cows.

ACKNOWLEDGMENTS

The authors wish to acknowledge the help of W. R. Pritchard, D. K. Sorensen and G. R. Spencer of the Veterinary Science Department for their part in the mastectomy operations.

REFERENCES

- (1) ADDIS, M. L'asportazione della mamella nella pecora, non influisce sul concepimento, sulla gravidanza e sull'involuzione uterina. *Proflassi*, 12: 169-172. 1939.

- (2) ALLCROFT, W. M. Observations on Some Metabolic Disorders of Cows as Evidenced by Chemical Analysis of Samples of Blood from Clinical Cases. (2) Some Aspects of Blood Chemistry. *Vet. J.*, 103: 30-47. 1947.
- (3) ALLEN, N. N. Blood Fat of Dairy Cattle. I. A Simple Volumetric Method for Determining Blood Fat. II. Factors Influencing the Fat Content of Blood Plasma. *Minn. Agr. Expt. Sta. Tech. Bull.* 130. 1938.
- (4) BARKEE, J. R. Blood Changes and Variations in the Female Bovine Toxemias. *Vet. Record*, 51: 575-580. 1939.
- (5) BERT, M. P. Sur l'origine du sucre de lact. *Compt. rend.*, 98: 775-777. 1884.
- (6) CLARK, E. P., AND COLLIP, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with Suggested Modification. *J. Biol. Chem.*, 63: 461-464. 1925.
- (7) CRAIGE, A. H., JR., AND STOLL, I. V. Milk Fever (Parturient Paresis) as a Manifestation of Alkalosis. *Am. J. Vet. Research*, 8: 168-172. 1947.
- (8) DRYERRE, H., AND GREIG, R. *Further Studies in the Etiology of Milk Fever*. Dumfries and Galloway, Vet. Med. Assoc. Oliver and Boyd, London. 1928.
- (9) EDDINGTON, E. H. A Study of the Effect of *Brucella abortus* in Udderless Cows. *J. Agr. Research*, 53: 225-233. 1936.
- (10) FISH, P. A. The Physiology of Milk Fever. II. *J. Am. Vet. Med. Assoc.*, 73: 10-37. 1928.
- (11) FISH, P. A. The Physiology of Milk Fever. III. The Blood Phosphates and Calcium. *Cornell Vet.*, 19: 147-160. 1929.
- (12) FISH, P. A. Milk Fever. 11th Int. Vet. Congress, 330-347. 1930.
- (13) FISH, P. A. Blood Phosphates in Milk Fever. *Proc. Soc. Exptl. Biol. Med.*, 26: 387-389. 1929.
- (14) FISKE, C. H., AND SUBBAROW, Y. The Colorimetric Determination of Phosphorus. *J. Biol. Chem.*, 66: 375-400. 1925.
- (15) FRANK, E. R. *Veterinary Surgery Notes*. Rev. Ed. Burgess Publishing Co., Minneapolis, Minnesota. Pp. 180-181. 1944.
- (16) GODDEN, W., AND ALLCROFT, W. M. Changes in the Composition of Cow's Blood at Time of Calving and a Comparison of the Blood of the Calf with that of its Dam. *Biochem. J.*, 26: 1640-1646. 1932.
- (17) GODDEN, W., AND DUCKWORTH, J. Variations in Serum Magnesium and the Partition of Serum Calcium in Normal Parturitions and Milk Fever. *Biochem. J.*, 29: 445-455. 1935.
- (18) GREIG, J. R. Studies in the Prevention of Milk Fever. *Vet. Record*, 10: 301-305. 1930.
- (19) GREIG, J. R. The Nature of Milk Fever. *Vet. Record*, 11: 148-152. 1931.
- (20) HIBBS, J. W., KRAUSS, W. E., POUNDEN, W. D., MONROE, C. F., AND SUTTON, T. S. Studies on Milk Fever in Dairy Cows. II. The Effect of Vitamin D on Some of the Blood Changes in Normal and Milk Fever Cows at Parturition. *J. Dairy Sci.*, 29: 767-782. 1946.
- (21) LITTLE, W. L. Milk Fever or Hypocalcemia of Parturient Cows. *Vet. Record*, 12: 683-686. 1932.
- (22) LITTLE, W. L., AND WRIGHT, N. C. The Aetiology of Milk Fever in Cattle. *Brit. J. Exp. Path.*, 6: 129-134. 1925.
- (23) LITTLE, W. L., AND MATTICK, E. C. V. The Calcium Content of Cows' Blood. II. (a) Calcium in the Blood of Parturient Heifers. (b) Effect of Injection of Parathyroid Extract, Irradiation, etc. (c) Some Case Reports of Milk Fever. *Vet. Record*, 13: 1091-1097. 1933.
- (24) MARSHALL, F. H. A., AND KIRKNESS, J. M. On the Formation of Lactose. *Biochem. J.*, 2: 1-6. 1907.
- (25) MOORE, B., AND PARKER, W. H. A Study of the Effects of Complete Removal of the Mammary Glands. *Am. J. Physiol.*, 4: 239-242. 1900.

- (26) NIEDERMEIER, R. P., AND SMITH, V. R. A Study of Some Blood Constituents of Cows Not Milked Following Parturition. *J. Dairy Sci.*, 31: 684-685. 1948.
- (27) PALMER, L. S., CUNNINGHAM, H. S., AND ECKLES, C. H. Normal Variations in the Inorganic Phosphorus of the Blood of Dairy Cattle. *J. Dairy Sci.*, 13: 174-195. 1930.
- (28) PORCHER, C. L'origine du lactose. *Arch. intern. physiol.*, 8: 356-391. 1909.
- (29) PRIBYL, E. Obsah elektrolytic v krevnim seru ovce a koz a v krevnim seru krav, při některých chorobach, zejména chorobach v době gravidity a, puerperia. *Zverol. Rozpravy Suppl. Zverol. Obzor.*, 7: 61-70; 73-78. 1933. (*Nutrition Abstracts & Revs.*, 4: 88. 1934-35.)
- (30) SCHULHOF, A. Hypoglykaemie a hypocalcaemie jako přicny puerperální paresy krav ve světle Klobonkovy contové theorie o pathogenese této choroby. *Klen. Spisy Skoly Zverol. Brno*, 10: 23-45. 1933. (*Nutrition Abstracts & Revs.*, 4: 699. 1934-35.)
- (31) SELLHEIM, H. Die marmäre Theorie über Entstehung des Eklampsiegiftes. *Zentr. Gynäköl.*, 34: 1609-1615. 1910.
- (32) SIMONSEN, D. G., WESTOVER, L. M., AND WERTMAN, M. The Determination of Serum Magnesium by the Molybdivanadate Method for Phosphate. *J. Biol. Chem.*, 169: 39-47. 1947.
- (33) SHATTOCK, L. G. The mammary glands and the foetus. *Lancet*, 83, II: 1838. 1905.
- (34) SJOLLEMA, B. Über die Bedeutung der Elektrolytkonstellation für den Organismus. Die Biochemie der Gebärpärese von Kühen. *Biochem. Z.*, 200: 300-308. 1928.
- (35) SJOLLEMA, B., AND SEEKLES, L. Über Störungen des mineralen Regulationsmechanismus bei Krankheiten des Rindes. (Ein Beitrag zur Tetaniefrage.) *Biochem. Z.*, 229: 358-380. 1930. (*Biol. Abs.*, 6: 3461. 1932.)
- (36) SMITH, V. R., AND BLOSSER, T. H. Parturient Paresis. I. The Incidence of Parturient Paresis and Changes in the Total Blood Serum Calcium at Parturition in Prepartum Milked Cows. *J. Dairy Sci.*, 30: 861-866. 1947.
- (37) WILSON, L. T., AND HART, E. B. The Chemistry of the Blood of Dairy Cows Before and After Parturition and its Relation to Milk Fever. *J. Dairy Sci.*, 15: 116-131. 1932.

CHANGES IN QUALITY OF CREAM MARKETED THROUGH BUYING STATIONS^{1,2}

T. J. CLAYDON AND W. H. MARTIN

Kansas Agricultural Experiment Station, Manhattan

In the last few years members of the butter industry have carried out extensive educational programs to encourage the production of better quality cream on the farm. The need for such improvement is generally recognized. Also, it usually is appreciated that under the average conditions involved in the marketing of cream through buying stations, changes in quality may continue until the cream is processed at the plant.

Manhart (6) studied the effect of the time element associated with marketing on the quality of cream in Indiana. He found that, under conditions of the study, butter made from cream 4 days or less old when sold by producers generally scored 1.22 points higher than butter from cream over 4 days old. He further found that an extended interval between delivery of cream to the station and processing at the creamery resulted in lower butter quality than when this interval was short. The higher quality cream deteriorated more than the lower quality cream during the period. The atmospheric temperatures involved were not indicated. Martin, Fay and Caulfield (7) showed that, although good cream held at 50° F. remained first grade for an average of 15 days, it became second grade in an average of 2 days when held at 90° F. Morrison, Nelson and Martin (8) found that the quality of cream decreased rapidly during holding in the cream station.

Over a period of years, various reports (2, 3, 4, 5) from the Dairy Commissioner, Kansas State Board of Agriculture, repeatedly indicated that a high percentage of cream in Kansas was of first grade quality when delivered by producers to stations. However, this percentage was much lower just prior to processing at the creamery. The reports indicated the need for getting cream to the churn with minimum deterioration and emphasized the necessity for reduction in the interval involved together with the employment of adequate cooling.

The problem of change in quality of cream between the time of purchase at stations and processing at creameries is recognized by members of the butter industry. Efforts have been made to improve the methods of handling cream during the period involved. Because of the importance of the quality problem and the necessity of directing efforts where they may be most effective, it is essential to know more specifically the extent of deterioration now occurring during the marketing intervals. The following study was undertaken to determine the quality of cream at the time of delivery to the buying station by Kansas pro-

Received for publication July 9, 1949.

¹ Contribution No. 187, Department of Dairy Husbandry.

² This study was supported by a grant from Swift and Co., Chicago, Ill.

ducers and the extent of any changes that develop under practical conditions until the cream is processed at the creamery.

METHODS

General procedure. The study was facilitated through the cooperation of a number of cream station operators and creamery representatives. It was carried out over a period of 1 year so that seasonal influences would be included. Cream buying stations were contacted in several areas to minimize any effect of regional quality. Although efforts were made to obtain representative cream stations, it is possible that those cooperating were more progressive than average. Since mechanical coolers are not common in stations in Kansas, only one station so equipped was included among those involved in the study.

Each station was visited on a day, usually Saturday, when cream receipts were expected to be relatively large so that the cream examined would represent a larger proportion of that marketed during a given period. Through the cooperation of the creameries involved, arrangements were made to re-examine the same cream at the creamery just prior to processing under prevailing plant schedules. In the summer, this usually was the day following purchase at the station. During winter, it was 1 to 3 days later. It is possible, in some instances, that the movement of cream from stations to creameries was more prompt on the days of sampling than at other times.

In all instances records were made of the general weather conditions on the days of sampling at the stations and at the creameries. Data were recorded on the weight, temperature and frequency of delivery of cream delivered by producers. At the creamery information relative to cream temperature, methods of handling, and the time interval between purchase from producers and processing in the plant was recorded.

Sampling procedure. A 4-oz. sample was taken directly from the well-stirred cream of each lot at the time of delivery by producers and this sample immediately was placed in ice water. The ladle used for sampling was rinsed in warm water and then in a hypochlorite solution (200-300 p.p.m.) after sampling each delivery to minimize contamination from one lot of cream to the next. After sampling, the cream delivered by the producers was dumped into 10-gallon cans in the usual manner followed in the buying stations. As each 10-gallon can was filled, a record was made of the individual deliveries contained. A sample then was taken from the full can in the same manner as from the separate lots of cream delivered by producers. The cans were tagged, using metal poultry seals, so that they could be identified subsequently at the creamery. All cream samples obtained were held iced and examined in the laboratory as promptly as possible.

After arrival at the creamery, and in accordance with the regular plant schedule, the cream from the tagged cans was re-examined and sampled just prior to processing. At this time the opinion of the butter-maker relative to scores and grades of the cream was noted. The samples were iced and returned to the laboratory for analyses. All sampling at stations and creameries was done by the same person so that methods and handling practices were consistent.

Quality determinations. Quality was determined on the basis of organoleptic tests supported by titratable acidity and formol titrations. Examinations were made on the samples representing individual deliveries and also on samples from the 10-gallon cans of cream as filled in the station, to indicate the quality of cream when first received. Similar tests were made on the samples obtained from the same 10-gallon cans of cream at the creamery, thereby showing the quality at the time of processing. Cream samples were scored for flavor according to the method commonly used in the butter industry where the numerical score is based on the estimated quality of the butter that should be obtained. Such flavor scores were used in preference to grades so that smaller differences in quality could be designated. Samples were scored in the laboratory by two experienced judges working independently. In the case of all samples obtained at creameries, the scores given by the butter-makers also were available for reference. Acidity determinations were made by titrating 9 g. of cream plus 9 ml. of distilled water with 0.1 *N* NaOH, using phenolphthalein as the indicator. Formol titrations were made by the method used by Martin, Fay and Caulfield (7) except that 2 ml. of formalin were used instead of 10 ml.

During the period of a year, 11 field trials were conducted involving 163 lots of cream delivered by producers to 9 stations. The number of deliveries examined at each station contact ranged from 7 to 21 and averaged slightly less than 15. The stations were located in eight towns in seven Kansas counties and shipped to seven different creameries in six counties. The distance that cream was shipped or hauled varied from 25 to 100 miles.

RESULTS

Cream as delivered to buying stations. The quality of farm-separated cream delivered to buying stations by producers varied over a wide range (table 1).

TABLE 1
Cream as delivered to buying stations by producers

Field trial	Month	Approx. outdoor temp. ^a		No. of deliveries	Weight of delivery		Flavor score	
		Range	Mean		Range	Av.	Range	Av.
		(° F.)	(° F.)		(lb.)	(lb.)		
1	June	50-64	57	21	10-82	35.5	89-92	90.8
2	June	64-80	72	7	13-40	30.3	88-91	90.2
3	July	68-84	76	15	5-40	20.6	89-91.5	90.2
4	Aug.	88-104	96	9	7-35	21.3	89-93	91.2
5	Sept.	72-102	87	20	11-47	23.6	88-91	90.2
6	Sept.	60-84	72	19	5-35	24.5	89-93	91.4
7	Nov.	32-54	43	20	4-42	17.6	90-92	91.2
8	Feb.	30-40	35	15	9-52	27.2	90.5-93	91.6
9	Apr.	60-84	72	18	8-79	35.3	90-92	90.9
10	Apr.	64-80	72	7	30-40	34.2	90-91.5	90.9
11	June	60-80	70	12	7-63	27.4	88-92	91.0
Summary		30-104		163	4-82	27.0	88-93	90.9

^a On day of sampling at station.

Study of the data involving the 163 individual deliveries showed that 82 per cent of the deliveries scored from 90 to 92 inclusive, with 13 per cent scoring below 90 and 5 per cent scoring above 92. Although varying amounts of deterioration had occurred in the cream on the farm, 58 per cent of the deliveries scored 91 or better.

In general, the cream was of lower quality during hot weather than during cool and moderate weather. When the mean outdoor temperature^a was above 70° F. on the days of sampling, the average score of the cream was 90.7 with 47

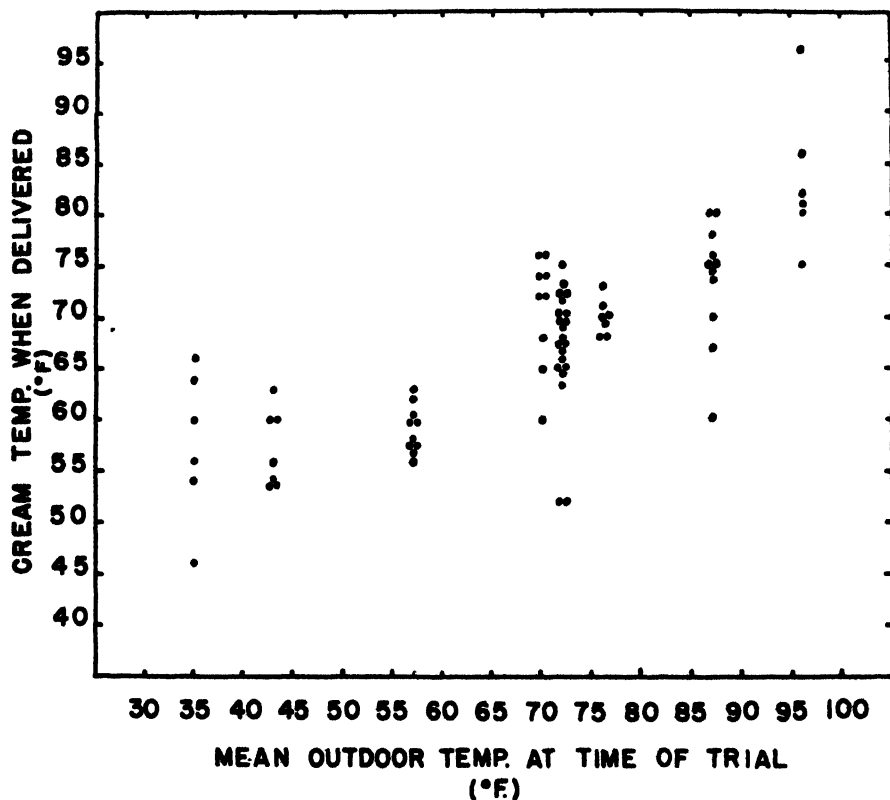


FIG. 1. Relationship of cream temp., when delivered, to mean outdoor temp. at time of trial. (77 deliveries.)

per cent of the deliveries scoring 91 or better and 18 per cent scoring below 90. When the mean outdoor temperature was 70° F. and below, the average cream score was 91.1 with 71 per cent of the deliveries scoring 91 or better and only 7 per cent scoring below 90.

Although temperature readings at the time of delivery were not obtained on all the cream, 77 of the deliveries ranged from 46° F. in cool weather to 96° F. in hot weather, with an average temperature of 67.4° F. The temperature of

^a Average of approximate minimum and maximum temperatures.

cream when delivered showed a general relationship with the mean outdoor temperature at the time (Fig. 1). An attempt was made to relate the temperature of the cream at the time of delivery to quality. However, the relationship was not particularly marked and was only significant during warmer weather. In trials when the mean daily outdoor temperature was above 70° F. the correlation coefficient was -0.337 for 45 deliveries (significant at 5 per cent level). Also, when the mean daily outdoor temperature was 70° F. and below, the relationship between cream temperature at the time of delivery and quality was non-significant, as indicated by a correlation coefficient of $+0.068$ for 32 deliveries.

There was no definite relationship between weight of delivery and quality at delivery. When the mean outdoor temperature was above 70° F. the correla-

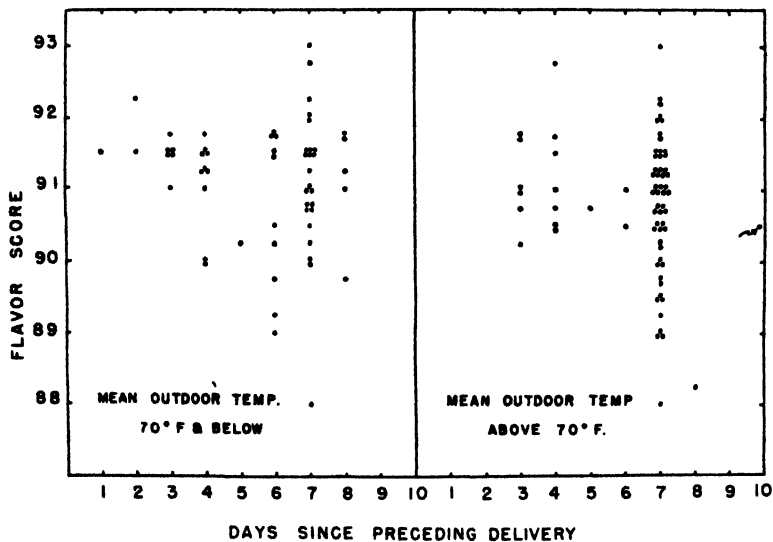


FIG. 2. Distribution of cream deliveries according to flavor score and frequency of delivery.

tion coefficient between weight and quality of 68 deliveries was -0.149 . When the mean outdoor temperature was 70° F. and below the correlation coefficient for 93 deliveries was $+0.168$.

The length of time since the previous delivery by the same producer (frequency of delivery) was obtained on 130 deliveries. This interval ranged from 1 to 10 days, with an average of 6 days. In 60 per cent of the cases the interval between deliveries was 7 days or more, indicating the tendency to make once-a-week delivery. An effort was made to determine the relationship between the frequency of delivery of the cream and its quality when sold. Since it seemed possible that the relationship might be closer during warmer weather than in cooler weather, the data on the deliveries were divided, as in previous correlations, into the group representing mean outdoor temperatures above 70° F. and the one representing temperatures of 70° F. and below. Figure 2 indicates that

there was no marked relationship between the frequency of delivery of the cream and its flavor score when delivered. For further verification, correlations were determined on the two groups. During the warmer weather, as might be expected, there was some tendency toward higher quality with more frequent delivery. In trials in which the daily temperature averaged above 70° F. there was a slight relationship between age and score for 72 deliveries, as indicated by a correlation coefficient of -0.276 , which is significant statistically. At mean outdoor temperatures of 70° F. and below, the coefficient for 58 deliveries was -0.165 , which is not significant. These values may have been influenced by the uneven distribution resulting from the larger number of deliveries at the 7-day period.

Cream as processed at creameries. During the interval between delivery of the cream at the station and processing at the creamery, there was definite deterioration in quality (table 2). The quality changes involved reductions in average flavor scores for each trial ranging from 0.3 to 1.2 points, with an over-all average decrease of 0.7 points. Changes in titratable acidities generally conformed to changes in flavor scores. Although changes in formol titrations occasionally were inconsistent, the average change was in accordance with other quality changes. The 61 individual 10-gallon cans of cream involved had a drop in score ranging from 0 to 2.0 points. Only three cans of cream had no change during the interval. Twenty-seven decreased 0.25 to 0.5 point in score, while 27 others lost 0.75 to 1.0 point. Four cans of cream dropped 1.25 to 2.0 points in score. The average loss in flavor score for the 61 cans was 0.7 points. The expected repeatability of these results is indicated by the fact that the true average loss in score probably lies between 0.5 and 0.8 points, as determined by 99 per cent fiducial limits.⁴

The amount of deterioration that developed in the cream during the interval between purchase at the stations and processing at creameries was correlated somewhat with the temperature of the cream just prior to processing. This was indicated by a correlation coefficient of $+0.295$ for 51 cans of cream on which temperatures were available (significant at 5 per cent level). However, this relationship was not particularly marked and probably was influenced by the different holding periods involved, possible temperature variations during the holding period and differences in the quality of the cream when purchased.

Except for trials 4 and 11 where some type of cooling was used, the cream temperature at the creamery varied generally with the outdoor temperature. The extent of deterioration tended to be related to the mean outdoor temperature. This was shown by a correlation coefficient of $+0.363$ for the 61 cans of cream involved (significant at 1 per cent level). Less deterioration usually occurred during the cooler months, even though the holding periods involved sometimes were longer. The greatest average loss in flavor score (1.2 points) occurred during the hottest weather (trial 4). Observations made during the study indicated that, in warm weather and without effective cooling, cream that was of high

⁴ Although it is doubtful that the losses in score follow closely a normal frequency distribution, it is believed that means of samples of 61 observations will do so, and hence the fiducial limits technique used here is satisfactory.

TABLE 2
Cream as collected at stations and as processed at creameries
(Cream accumulated in 10-gallon cans)

Field trial	Month	Approx. ^a outdoor temp. range	No. of 10-gal. cans	Method of cooling		Av. cream, temp. at creamery	Approx. ^b interval involved	Av. quality					
				Station	Creamery			Flavor score		Titratable acidity		Formol titration	
								At stations ^c	At creamery ^d	At stations ^c	At creamery ^d	At stations ^c	At creamery ^d
(° F.)													
1	June	50-70	9	none	none	56	20	90.9	90.2	0.59	0.68	3.0	2.5
2	June	64-80	4	none	none	72	36	90.3	89.5	0.64	0.84	2.9	3.3
3	July	68-90	4	none	none	77	20	89.8	89.0	0.92	1.10	3.0	3.7
3	Aug.	88-104	3	none	spray	75	20	91.4	90.2	0.62	0.83	2.4	2.6
5	Sept.	72-102	6	not used	none	84	20	89.8	88.9	0.82	1.15	3.4	4.0
6	Sept.	60-84	6	none	none	72	20	91.5	90.7	0.68	0.81	3.0	2.6
7	Nov.	32-54	6	none	not used	58	40	91.6	91.0	0.53	0.60	2.3	2.5
8	Feb.	28-40	6	none	not used		40	91.5	91.0	0.45	0.62	2.0	2.2
9	Apr.	60-84	9	not used	not used	74	20	91.1	90.4	0.52	0.63	2.8	3.1
10	Apr.	64-80	4	not used	not used		24	91.2	90.6	0.57	0.66	2.5	2.7
11	June	60-80	4	"walk-in"	"walk-in"	45	68	91.2	90.9	0.53	0.64	2.4	2.9
Summary			61			68.1	29.8	90.9	90.2	0.62	0.78	2.7	2.9

^a During period from purchase at station to processing at creamery.

^b Interval between purchase at station and processing at creamery.

^c At time of purchase.

^d At start of processing.

quality at the time of delivery deteriorated proportionately more during the marketing interval than did the lower quality cream.

A number of stations and most creameries were equipped with some facilities for spray-type cooling. However, these generally were not in use on the days that cream was sampled and examined, even when weather conditions would have justified such use. In trial 11, where "walk-in" type refrigeration was provided at both the station and the creamery, the smallest average amount of deterioration (0.3 points) occurred, even though the interval from purchase to processing was 68 hours and the mean outdoor temperature was 70° F.

DISCUSSION

In general the quality of cream delivered to buying stations by producers under Kansas conditions was somewhat higher than anticipated. On the other hand it was evident that a number of producers were marketing cream of inferior quality. The lack of any striking relationship between quality and the size or frequency of delivery is difficult to explain since the temperature of the cream at the time of delivery to the station seldom indicated thorough cooling practices on the farm. Nevertheless, the relationship between the temperature of cream when delivered and its quality was not particularly marked. Cream temperature at the time of delivery, however, is not necessarily indicative of cooling practices followed throughout the entire accumulation period on the farm. It should be noted that the results were obtained under actual commercial conditions and where cream holding and handling practices on farms were neither controlled nor known.

In most trials, cream was moved from the stations to the creameries and processed with a minimum of delay. In some cases the fact that the study was being made may have prompted more rapid movement to and earlier processing at the creamery. It also is possible that standards of operation in the stations contacted were above average, since such frequently is a characteristic where buyers are willing to cooperate. Accordingly, it is considered that the results on the amount of deterioration during the interval between purchase from the producer and processing at the creamery give a conservative measure of the quality changes under average commercial conditions. In general, the change in quality that occurred during the marketing interval would be expected to result in butter of from 0.5 to 1.0 points lower in score than would be warranted by the quality of the cream delivered by producers.

The observation that deterioration was relatively greater in the higher quality cream, in the absence of special precautions, is in accordance with the results of a previous study (1) and generally agrees with the findings of Manhart (6). Such a situation deserves special attention in planning for improvement in cream quality. It emphasizes the fact that any improvement in the quality of cream marketed by producers must be accompanied by corresponding improvement in subsequent handling methods if maximum benefits are to be realized.

The general failure to provide adequate cooling for cream subsequent to purchase from producers undoubtedly is a factor in the quality problem. The fact

that least deterioration occurred where cooling practices were applied most conscientiously and in the face of the longest holding period (trial 11) would suggest that adequate cooling is an important factor in the cream station method of marketing. Although air temperatures in the "walk-in" coolers were not obtained and air cooling usually has not been advocated as satisfactory for cream, the results indicated that even this type of cooling was beneficial in the case involved.

In quality improvement programs, attention must be given to the phase of marketing subsequent to delivery of cream by producers as well as to production on the farm. It would appear that more rapid initial progress would be obtained from efforts so directed than from the more extensive efforts necessary to obtain results with widely scattered producers.

SUMMARY AND CONCLUSIONS

A study was made of the changes in quality of farm-separated cream from the time of delivery to buying stations until just prior to processing at creameries. The cream involved 163 deliveries to nine stations through different seasons of the year and was generally representative of cream so marketed in Kansas. From the stations the cream was shipped from 25 to 100 miles to seven different creameries.

Although some of the cream was of relatively high quality at the time of delivery by producers, the average quality and the wide limits of variation showed that deterioration had occurred in cream on the farm. There was no close relationship between cream quality and weight of delivery. The correlations between quality and frequency of delivery and between quality and cream temperature at the time of delivery were low even in warmer weather and not significant in cooler weather.

Definite deterioration occurred in the cream between the time of purchase from the producer and processing at the creamery. Decreases in flavor scores of individual 10-gallon cans of cream ranged from 0 to 2.0 points. In the average scores of cream in each trial, the losses ranged from 0.3 to 1.2 points, with an over-all average loss of 0.7 points. Deterioration generally was greater in warmer weather than in cooler weather and was relatively more extensive in the higher quality cream.

With a few exceptions, cooling procedures were not generally in use in either stations or creameries on the days that cream was sampled and examined, even in warm weather. The limited amount of deterioration that occurred in the case where mechanical cooling was used at both station and creamery suggested the importance of effective cooling in the marketing of cream through stations.

In cream quality programs, besides emphasizing the production phase, consideration must be given to controlling deterioration subsequent to the delivery of cream to stations.

ACKNOWLEDGMENT

The authors are grateful to H. C. Fryer, statistician, Kansas Agricultural Experiment Station, for suggestions and assistance in the statistical treatment of data.

REFERENCES

- (1) CLAYDON, T. J. AND MARTIN, W. H. Final Cream Quality Resulting from Keeping Deliveries Segregated Versus Mixing as Practiced in Buying Stations. *J. Dairy Sci.*, **32**: 534-542. 1949.
- (2) DODGE, H. E. Kansas Cream Improvement Campaign. Mimeo. Rept., Dairy Div., Kan. State Board of Agr. 1937.
- (3) DODGE, H. E. Kansas Dairy Products Quality Improvement Campaign. Mimeo. Rept., Dairy Div., Kan. State Board of Agr. 1941.
- (4) DODGE, H. E. Dairy Division Report. Kansas State Board of Agriculture. Mimeo. 1943.
- (5) DODGE, H. E. Research Project on Cream Quality. Mimeo. Rept., Dairy Div., Kan. State Board of Agr. 1948.
- (6) MANHART, V. C. Effect of the Time Element in Marketing Cream for Buttermaking on Quality. *Purdue Agr. Expt. Sta. Bull.* 383. 1933.
- (7) MARTIN, W. H., FAY, A. C., AND CAULFIELD, W. J. The Effect of Temperature and Time of Storage of Cream on the Rate and Type of Deterioration. *J. Dairy Sci.*, **20**: 667-678. 1937.
- (8) MORRISON, R. W., NELSON, F. E., AND MARTIN, W. H. Effect of Holding Cream in the Buying Station upon the Mold Content and Certain Other Quality Factors. *J. Dairy Sci.*, **25**: 195-202. 1942.

THE KEEPING QUALITY OF PASTEURIZED MILK IN HOME REFRIGERATORS¹

S. J. WEESE AND H. O. HENDERSON

Department of Dairy Husbandry, West Virginia University, Morgantown

It generally is agreed that the practice of every other day delivery of milk that was started during World War II reduced the cost of delivery so much that most distributors prefer to retain the economy of this practice.

With delivery either every other day or 3 days a week the milk must retain its quality up to 4 days after pasteurization. To justify such delivery the consumer must be assured that the product delivered will retain its high quality for at least these 4 days. For this reason, a study was undertaken to determine if good pasteurized milk would retain its high quality when delivered every other day or 3 days a week and kept in home refrigerators under ordinary home conditions.

Several investigations have shown that good quality milk, properly processed, can be kept for a considerable length of time in household refrigerators under laboratory conditions. Very few of these, however, have had the milk subjected to conditions in the ordinary home refrigerators.

Dahlberg (3), in a laboratory study that was somewhat similar to home conditions, made in the New York metropolitan area in 1944 and 1945, showed that the keeping quality of pasteurized milk was good enough at all seasons of the year to permit every other day delivery without impairment of milk quality. He concluded, however, that to insure good keeping quality, milk should not be stored above 50° F.

Burgwald and Josephson (2), found "that milk of good quality can be expected to retain excellent bacteriological and flavor qualities for at least 4 days during the summer months and 6 to 7 days during winter months if refrigerator temperatures are maintained near 40° F."

METHODS

Sixteen families with electric or gas refrigerators were used as cooperators. They were furnished with quart bottles of ordinary pasteurized milk of the same quality as that sold in the retail store at the University creamery. The milk was cooled immediately after milking and the evening milk was kept in the cooler at the dairy farm and delivered to the University creamery the following morning with the morning's milk. It then was pasteurized (144° F. for 30 min.) and bottled and held in the cooler until the next morning before delivering, as this is the normal procedure in most milk plants.

The quart bottles of milk either were delivered to the homes in the same manner as used by milk plants, or the cooperators secured the milk directly from

Received for publication July 11, 1949.

¹ Published with the approval of the director, West Virginia Agricultural Experiment Station, as Scientific Paper no. 410.

TABLE 1

Summary of the flavor scores of 207 samples of milk left in 16 home refrigerators from 3 to 7 days under summer conditions

	Elapsed time				
	3 d.	4 d.	5 d.	6 d.	7 d.
No increase ^a	36	98	43	18	12
Av. flavor score					
Test samples	38.0	38.0	35.5	35.3	34.1
Control samples	39.2	39.0	38.2	38.8	37.5

the creamery. Some of the commuter cooperators lived as far as 20 miles distant from the creamery, in which case the milk received no refrigeration enroute to the homes. The cooperators were asked to use about three-fourths of the milk and to treat this bottle of milk in the same manner as their regularly delivered milk, such as bringing it out at mealtime, shifting it around in the refrigerator, etc. The remainder, or approximately one-fourth (usually less) of the original milk was returned to the laboratory and scored for flavor by a committee of three or more members of the dairy staff. This committee also scored the fresh milk for flavor the day it was delivered.

A bacterial count of the milk was made the day it was delivered and again when the samples were brought from the cooperators at the end of each test period. The plate colony count method was used, as outlined by the American Public Health Association (1). An acidity test, titrating with N/10 sodium hydroxide, with phenolphthalein as an indicator, was made on the milk the day it was delivered and, likewise, when it was brought back to the laboratory from the cooperators. A thermometer was placed in each of the refrigerators and occasional readings made to see if the temperature was within the normal range.

In all, 207 quarts of milk were distributed during the months of May and June, 1946, and June, July and August, 1947. The time of the study, therefore, represented summer weather conditions. The length of time the milk was left with the cooperators ranged from 3 to 7 days. The following flavor scorecard was used to score the returned samples:

TABLE 2

The percent of total samples that showed varying degrees of increase in acidity (calculated as lactic acid) of milk in home refrigerators from 3-7 days after delivery as compared with acidity on delivery date

	% of samples showing indicated increase after an elapsed time of:									
	3 d.	con ^b	4 d.	con	5 d.	con	6 d.	con	7 d.	con
No increase ^a	83.3	100	74.0	80	33.3	25	30.0	33	8.3	
Slight increase	13.9		23.0	20	31.2	75	25.0	66	33.3	100
Moderate increase	2.8		2.0		13.3		10.0		25.1	
Great increase	0.0		1.0		22.2		35.0		33.3	

^a Key to acidity increases: No increase—0.0 to 0.005% lactic acid; slight increase—0.005 to 0.015% lactic acid; moderate increase—0.015 to 0.025% lactic acid; great increase—0.025 and up percent lactic acid.

^b Con. = control.

40 —no criticisms

38-40—slightly to moderately off-flavored (good)

35-38—distinct off-flavor, distinct absorbed flavor, definitely unpalatable, but still usable (fair)

25-35—high acid, bitter, stale, old. Very pronounced off- or absorbed flavor, generally not usable as a beverage (poor)

A quart bottle of milk was placed in the laboratory refrigerator to serve as a control at the time of delivery of the milk to the cooperators. The refrigerator temperature was maintained at 40° F. and the milk bottles were not uncapped until time to be scored for flavor. The results of the flavor score are given in table 1. The figures indicate that good quality milk will retain a good flavor 3 to 4 days after delivery, but after the fourth day there is a decided drop in flavor.

TABLE 3

Number of samples and percent of total samples that showed varying degrees of increase in bacteria count (by the plate count method) of milk stored from 3 to 7 days in home refrigerators over the bacteria count of the same milk on delivery date

	Elapsed time									
	3 d.		4 d.		5 d.		6 d.		7 d.	
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>Test samples</i>										
No increase ^a	15	42.8	16	16.8	5	11.4	0	0.0	0	0
Slight increase	8	22.9	26	27.4	12	27.3	2	9.5	0	0
Moderate increase	4	11.4	15	15.8	10	22.7	4	19.1	0	0
Great increase	8	22.9	38	40.0	17	38.6	15	71.4	12	100
Total test samples	35		95		44		21		12	
<i>Control samples</i>										
No increase	3	100	3	33	1	25	1	33		
Slight increase	0	0	3	33	2	50	1	33	1	100
Moderate increase	0	0	1	11	0	0	1	33		
Great increase	0	0	2	22	1	25				
Total control samples	3		9		4		3		1	

^a Key to increases—compared with count the day milk was given to cooperators: No increase—no counts higher than original milk; slight increases—1 to 5 times higher than original milk; moderate increases—5 to 15 times higher than original milk; great increase—15 times or higher than original milk

Of the 3 and 4 day old milk, 97 per cent had none or a slight increase in acidity, while with 5 and 6 day old milk only 64.4 and 55 per cent, respectively, had none or a slight increase. The results from the acidity study are given in table 2.

The number of samples and the percentage increase in bacteria counts in various amounts is given in table 3. The arithmetic average of the initial plate counts was 6,500. The plate counts of returned samples from cooperators showed such a wide variation due to different methods of handling the milk that tabulation would have but little value, but the average percentage increase by days is given. There was a decided increase in bacteria count of 5 and 6 day old milk over the 3 and 4 day old milk.

DISCUSSION

Considerable difficulty was experienced by the committee in determining the flavor score of returned samples in several instances. This was due mostly to absorbed refrigerator flavors. In some cases the resultant flavor was not too objectionable to some of the committee, while with others it was more serious. This partly was solved by setting up a flavor scorecard as previously mentioned.

As might be expected, there was a wide variation in the way the milk was handled by the cooperators. Certain cooperators were careless in promptly refrigerating the milk and seeing that the cap was put back on the bottle after part of the milk was used. Uncapped milk and caps improperly replaced were responsible for most of the absorbed flavors of the returned samples. In a few cases the committee could tell what product was stored in the refrigerator. In most cases, however, their remarks were "off flavor", "absorbed flavor", or "fruity flavor".

The length of time the milk was without refrigeration when delivered to the cooperators was from 1 to 3 hr. and a period of time somewhat shorter was used in returning the unused portion for laboratory examination. The keeping quality of the milk that was delivered 20 miles from the creamery compared favorably with the milk delivered a few hundred yards. This probably was due to efficient refrigeration and care by the cooperators.

The average temperature of the refrigerators varied from 37 to 52° F., with the higher temperature refrigerators permitting the greatest increase in plate count on the returned samples. Some of the cooperators had consistently higher plate counts and lower flavor scores on returned samples which were not justified by refrigerator temperatures. Some carelessness of the cooperators was found in replacing milk caps on bottles of milk partially used, resulting in lower flavor scores due to absorbed flavors. "Good housekeeping" in the refrigerator is important in keeping the milk free from absorbed flavors.

The control samples kept in the laboratory refrigerator (40° F.) scored consistently higher in flavor and increased less in acidity and bacteria count than did the milk returned from the cooperators as would be expected. Part of the control samples maintained their original flavor score as scored by the committee, but a lower flavor score in some cases was due to a lack of that fine clean flavor of the fresh milk. The committee did not try to differentiate between absorbed and developed flavors but many of the returned samples were criticized as "fruity".

The highest score given any sample of milk was 40 (no criticism). Of the 20 samples of fresh milk scored by the committee, 17 scored 40, 1 scored 39.5 and 2 scored 39 on flavor.

CONCLUSIONS

Delivery either every other day or three times a week is sufficient for pasteurized milk of good quality, provided the distributor and consumer use reasonable care in handling the product.

Consumer education is needed to aid the family in getting a better appreciation of how to care for the milk after it is delivered.

REFERENCES

- (1) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Dairy Products. 8th Ed. Am. Pub. Health Assoc., New York, Pp. 7-40. 1941.
- (2) BURGWARD, L. H., AND JOSEPHSON, D. V. The Effect of Refrigerator Storage on the Keeping Qualities of Pasteurized Milk. J. Dairy Sci., 30: 383. 1947.
- (3) DAHLBERG, A. C. The Keeping Quality of Pasteurized Milk. N. Y. (Cornell) Univ. Agr. Expt. Sta. Bull. 839. 1946.

THE EFFECT OF PENICILLIN UPON THE FERTILITY OF SEMEN FROM RELATIVELY INFERTILE BULLS¹

JOHN O. ALMQUIST

Department of Dairy Husbandry, The Pennsylvania State College, State College

In attempts to control the bacteria which are present in bull semen, it was found that penicillin (2) retarded bacterial growth at levels which did not exert an injurious effect upon the livability of the spermatozoa. The first experiment to determine the effect of penicillin upon fertility (1) showed that the addition of either 500 or 1,000 units of penicillin per ml. of diluted semen did not affect significantly the fertility of semen from bulls of relatively high breeding efficiency. Since it has been suggested that semen from bulls of lowered fertility might contain types of bacteria associated with breeding difficulties (3, 4), the present study was conducted to test the effect of penicillin upon the fertility of semen from relatively infertile bulls used in routine artificial breeding.

EXPERIMENTAL

In cooperation with the Western Pennsylvania Artificial Breeding Cooperative, Clarion, Pa., four Guernsey bulls and one Holstein bull of relatively low fertility were selected. Four concentrations of penicillin, with appropriate controls, were compared using a Latin square experimental design. Levels of 250, 500, 750 and 1,000 Oxford units of penicillin per ml. of yolk-citrate diluter were chosen, as a previous study (2) had shown that these amounts were not toxic to spermatozoa during the time which semen routinely is used in artificial breeding. The 5×5 Latin square experiment was repeated three times. The first experiment began December 11, 1946, and the last replicate was completed June 27, 1947. Semen samples were collected from each bull once approximately every 10 days and this interval of time was considered the collection period. During each collection period all treatments were used but the semen from a bull received only one of the five possible treatments.

The crystalline sodium salt of penicillin was dissolved in sterile 3.6 per cent sodium citrate dihydrate buffer. When the design of the experiment involved the addition of penicillin, the desired amount of penicillin solution and citrate buffer were mixed with egg yolk so as to ensure the preparation of a diluter which consisted of a 1:1 ratio of yolk to buffer. However, since the completion of the experiment it has been found that this particular precaution was unnecessary.

All semen was diluted at a fairly constant, though relatively low, rate as indicated by the fact that 70 of the 100 semen samples used in the study were diluted at the rate of 1:10. The average dilution rate was 1:11, with a range of from 1:10 to 1:16. None of the diluted semen was used for insemination on the day

Received for publication July 11, 1949.

¹ Authorized for publication June 20, 1949, as paper no. 1526 in the Journal Series of the Pennsylvania Agricultural Experiment Station. The penicillin was provided by Charles Pfizer and Co., Inc., Brooklyn, New York.

TABLE 1
Fertility of bulls during 6-mo. period prior to experiment

Bull	No. 1st and 2nd services	% non-returns after		Decrease in % non-returns (correction factor)
		30 to 60 d.	90 to 120 d.	
1 G	315	56.2	48.3	- 7.9
2 G	470	61.1	50.2	-10.9
3 G	194	50.5	28.9	-21.6
4 G	595	49.1	30.8	-18.3
5 H	489	50.9	30.3	-20.6

of collection so that the penicillin had ample time in which to exert its effect upon bacteria present in the samples.

The fertility of the five bulls during the 6-mo. period immediately preceding the experiment is shown in table 1. While the per cent 90- to 120-day non-returns for each bull is at or below 50 per cent, three bulls exhibited notably large correction factors when comparing 30- to 60-day with 90- to 120-day non-returns. Each of the bulls was producing semen which was average or above in both initial motility and concentration at the time of selection. During the experiment the average motility was 66 per cent. Only two semen samples (bull 1 G) exhibited motility as low as 50 per cent, and the remaining 98 samples showed either 60 or 70 per cent actively motile spermatozoa at the time of collection.

Average response in fertility to different levels of penicillin. Table 2 shows the average fertility data for the four levels of penicillin. The per cent non-returns for each individual treatment represent a mean of 20 ejaculates. Analysis of variance of the per cent 6-mo. non-returns for 3,576 first and second services demonstrated that the 500 and 1,000 unit levels of penicillin brought about highly significant ($P = < 0.01$) increases in fertility when compared with the untreated controls. While the 250 and 750 unit levels each increased the apparent conception rate by an average of 8.7 percentage units, the improvement was not statistically significant. The differences, however, did approach significance at the 5 per cent level of probability. The differences in breeding efficiency among the five bulls were highly significant although the interaction of treatments and bulls was not significant even at the 5 per cent point. The latter suggests that the semen of the various bulls reacted in a similar manner when treated with penicillin.

TABLE 2
Effect of penicillin upon the fertility of semen from five relatively infertile bulls

Units of penicillin/ml. of diluter	No. 1st and 2nd services	% non-returns after 6 mo.	Improvement over controls
0	671	48.0	
250	749	56.7	+ 8.7
500	677	61.4	+ 13.4
750	757	56.7	+ 8.7
1000	722	63.3	+ 15.3
Combined levels of penicillin	2905	59.4	+ 11.4

Greatest over-all improvement in fertility was obtained with 1,000 units of penicillin per ml. of diluter. As shown in table 2, the difference in favor of this level of penicillin was 15.3 percentage units, i.e., 15.3 per cent more cows apparently conceived than when untreated yolk-citrate diluted semen was used for insemination.

To compare the over-all results of adding penicillin at either 250, 500, 750 or 1,000 units per ml. of diluter with the results obtained where no penicillin was added, the data for the four levels of antibiotic were combined. Of the 2,905 cows inseminated with penicillin-treated semen, there was an average improvement in apparent conception rate of 11.4 percentage units as compared to the 671 cows inseminated with diluted semen containing no penicillin.

Variations in response of the individual bulls. As might be anticipated, an examination of the data showed wide variation in the response of the individual bulls when the semen was treated with penicillin. Since the addition of 1,000 units of penicillin per ml. of diluter resulted in the largest improvement in fertility, this level was compared to the untreated controls to show the variation by

TABLE 3
The response in fertility of the semen by individual bulls

Bull	Units of penicillin/ml. of diluter	No. 1st and 2nd services	% non-returns after 6 mo.	Improvement over controls
1 G	0	105	39.0	
	1000	86	39.5	+ 0.5
2 G	0	120	49.2	
	1000	102	63.7	+ 14.5
3 G	0	101	33.7	
	1000	98	65.3	+ 31.6
4 G	0	166	63.3	
	1000	224	67.4	+ 4.1
5 H	0	179	46.4	
	1000	212	67.5	+ 21.1

bulls. These data are presented in table 3. Treated semen from three of the five bulls showed large increases of 14.5, 21.1 and 31.6 percentage units over the controls. The other two bulls showed only small increases of 0.5 and 4.1 percentage units. There appeared to be little uniformity in the magnitude of the response even among the three bulls which showed greatest improvement. This may be attributed in part to the relatively small number of inseminations in some instances as the inseminators preferred to use semen from bulls of relatively high fertility which was available at the same time.

It will be noted in table 3 that the untreated diluted semen from all of the bulls but one (4G) was below 50 per cent 6-mo. non-returns. However, the control semen for this bull averaged 63.3 per cent or more than double his average of 30.8 per cent 3-mo. non-returns for the period prior to the experiment (see table 1). Based on the control data, therefore, this bull should not be considered a relatively infertile bull. The reason for this marked improvement in fertility is not known. Thus, in reality, only the semen from one of the four bulls (1 G) failed to respond to treatment with penicillin.

DISCUSSION

The results of this experiment indicate that the addition of penicillin to diluted semen offers a means of improving the breeding efficiency of certain bulls of lowered fertility. That this treatment definitely should not be considered as a cure-all for breeding difficulties with bulls is shown by the failure of one bull to respond, along with the variability in the degree of response of the three bulls showing large increases in fertility.

The 13-yr.-old bull, 1 G, which showed no significant improvement in fertility, illustrates a case in which penicillin treatment of the semen was not beneficial. Toward the end of the experimental period and up to the time of slaughter 7 mo. after the experiment, the fertility of this bull showed a very definite decline. Histological examination of the testes revealed marked degeneration of the seminiferous tubules.

At the time this study was initiated laboratory facilities were not available for bacterial analysis of the treated and untreated semen. While it is possible that the large increases in fertility exhibited by three of the five bulls were due to control of certain harmful types of bacteria, the actual reason for the beneficial effects is not yet known. Further fertility studies with additional relatively infertile bulls are now being completed using penicillin and streptomycin alone and in combination. In addition, bacteriological investigations are in progress to determine whether the beneficial effects of penicillin reported here may be attributed to bacterial control. For the present, the semen from each bull of low fertility will have to be tested by the trial and error method to determine whether the addition of penicillin is of value in improving fertility.

SUMMARY

Penicillin was added to the semen of five relatively infertile bulls at the rate of 250, 500, 750 and 1,000 units per ml. of diluter. Based on 3,576 inseminations, levels of penicillin of 500 and 1,000 units brought about highly significant increases in fertility of 13.4 and 15.3 per cent of the cows inseminated, respectively. The 250 and 750 unit concentrations each showed average increases of 8.7 percentage units over the controls and these differences approached significance. Greatest improvement in breeding efficiency was obtained with 1,000 units of penicillin per ml. of diluter.

The variation in results among the bulls indicated that penicillin had a very beneficial effect upon the semen from certain bulls of lowered fertility, while failing to be of significant value when added to the semen of other bulls. Thus, three of the five bulls showed large increases in fertility of 14.5, 21.1 and 31.6 percentage units when 1,000 units of penicillin were added per ml. of diluter. Of the remaining bulls, one showed a small increase of 4.1 per cent while the other showed no beneficial response.

ACKNOWLEDGMENTS

The author is indebted to R. C. Miller of the Department of Agricultural and Biological Chemistry for his suggestions concerning the statistical analysis

of the data and to George W. Thompson, Manager of the Western Pennsylvania Artificial Breeding Cooperative for carrying out the details of the field trial.

REFERENCES

- (1) ALMQUIST, J. O., THORP, W. T. S., AND KNOTT, C. B. The Effect of Penicillin upon the Livability, Fertility and Bacterial Content of Bull Semen. *J. Animal Sci.*, 5: 400-401. 1946.
- (2) ALMQUIST, J. O., THORP, W. T. S., AND KNOTT, C. B. The Effect of Penicillin upon the Livability, Glycolysis, and Bacterial Content of Bovine Semen. *J. Dairy Sci.*, 31: 11-19. 1948.
- (3) GUNSALUS, I. C., SALISBURY, G. W., AND WILLETT, E. L. The Bacteriology of Bull Semen. *J. Dairy Sci.*, 24: 911-919. 1941.
- (4) PRINCE, P. W., ALMQUIST, J. O., AND REID, J. J. Bacteriological Studies of Bovine Semen. II. The Incidence of Specific Types of Bacteria and the Relation to Fertility. *J. Dairy Sci.*, 32: In press. 1949.

THE RELATION OF THE CAROTENOID AND VITAMIN A CONTENT OF SUMMER MILK TO THE CAROTENOID CONTENT OF THE PASTURE HERBAGE

J. W. HIBBS, W. E. KRAUSS AND C. F. MONROE

Ohio Agricultural Experiment Station, Wooster

Numerous reports have established the fact that the vitamin A activity of butterfat follows a definite seasonal trend. In northern climates, the highest values are obtained in summer when green forage is abundantly available with the concentration of carotene and vitamin A decreasing progressively throughout the winter season, the degree depending largely upon the carotenoid content of the roughage consumed (2, 4, 5, 6, 7, 9, 10).

Mitchell and Wise (8) have reported the effects of continual and rotational pasturing of Bermuda grass on the carotene content of the pasture grass and the carotene content of the milk produced therefrom. No marked effect of the systems of grazing was reflected in the carotene content of the herbage or in the carotene content of the milk.

At the Ohio Agricultural Experiment Station on a 60-acre farm devoted entirely to pasture research, a herd of Jersey cows has been used to study various methods of pasturing permanent bluegrass pastures and legume mixtures. As an integral part of this pasture research program, a study was made of the relation of the carotenoid content of the pasture grasses to the carotenoid and vitamin A content of the milk produced on these pastures. The results of this study are presented in this report.

EXPERIMENTAL

During the pasture seasons of 1945, 1946 and 1947, three groups of Jersey cows were used in the pasture experiments. In each pasture period one group (A) was pastured on permanent bluegrass without supplemental hay and another group (B) ate the same pasture plus supplemental hay, fed *ad libitum* in the barn during the time the cows were being milked. A third group (C) was pastured on various legume mixtures without supplemental hay. The bluegrass pastures were well fertilized and contained varying amounts of white clover, depending on the season and weather conditions.

Each year in April, just before the cows were turned out to pasture, a sample of milk was collected from the individual cows of each pasture group. Subsequently, during each pasture period throughout the pasture season, individual milk samples were collected and composited according to morning and evening production and analyzed for vitamin A and carotenoids. Sampling was done as near to the middle of the pasture period as could be anticipated.

Received for publication July 11, 1949.

About 4 days prior to the milk sampling date, a "plucked" sample of pasture herbage was taken from the various pasture plots on which the cows were grazing. Information concerning the length of the pasture periods, cow groups, numbers of cows in the groups, description of the pastures, date of milk sampling and date of pasture sampling is indicated in fig. 1 (1945), fig. 2 (1946), and fig. 3 (1947) and the accompanying legends.

Milk carotenoids and vitamin A were determined essentially by the method described by Boyer *et al.* (1), except that hot saponification was used instead of cold. The carotenoids in the pasture were determined according to a procedure developed in the authors' laboratory as a composite of several methods. The

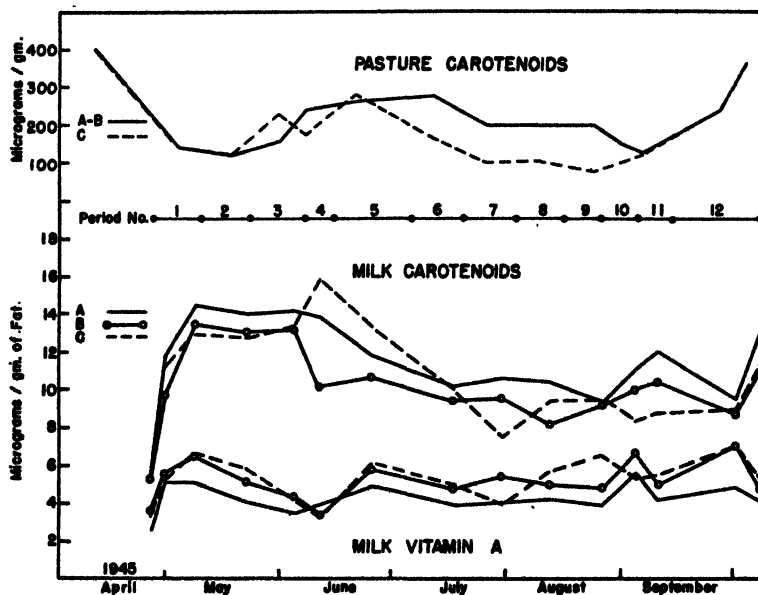


FIG. 1. The relation of the carotenoids in the pasture to the carotenoids and vitamin A content of the milk, 1945. Groups A and B were pastured on bluegrass during periods 1, 2, 3, 4, 5, 6, 7, 8, 9 and 12, on orchard grass in period 10, and on alfalfa-timothy in period 11. Group C was pastured on bluegrass during periods 1, 2 and 12, on alfalfa-timothy in periods 3, 4, 6, 7, 8 and 9, on orchard grass in period 5, and on alfalfa-bromegrass in periods 10 and 11. No. of cows per group was 5.

fresh pasture samples are chopped finely in a Hobart food chopper and a 10-gm. aliquot immediately is weighed, placed in a 250-ml. beaker and covered with acetone. Usually these samples are allowed to stand overnight in a cooler maintained at 40° C; this extracts a large proportion of the pigments. Two 100-g. samples are weighed for the dry matter determination at the same time.

The supernatant acetone then is decanted through a filter paper into a 500-ml. separatory funnel. The sample remaining in the beaker is transferred to a Waring blender and a foam-producing mixture of 2 parts acetone to 1 part

petroleum ether (B.P. 35-60) is added in an amount sufficient to cover the blades of the blender. If a dense foam does not develop when the blender is started, the addition of a few drops of water usually produces the desired results. The foam prevents splashing and possible loss of sample. After about 5 min. of grinding, the extract is decanted through the filter paper and the extraction procedure is repeated on the residue until the green color is extracted. This usually is complete after three to four times.

The petroleum ether fraction containing the green and yellow pigments is separated from the combined extracts by the addition of water to the separatory funnel. The bottom layer containing the acetone is drawn off and the upper

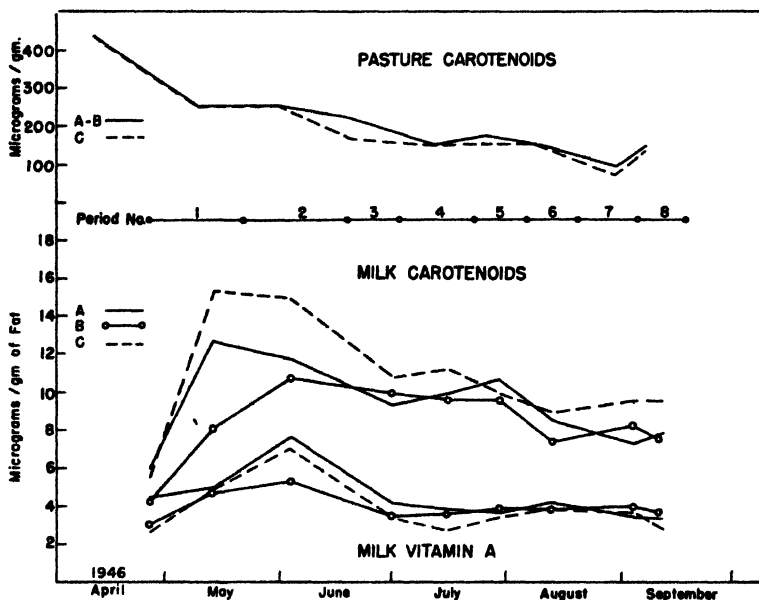


FIG. 2. The relation of the carotenoids in the pasture to the carotenoid and vitamin A content of the milk, 1946. Groups A and B were pastured on bluegrass during periods 1, 3, 5 and 7, on a legume mixture consisting of alfalfa with timothy, brome-grass and ladino clover in periods 2, 4 and 6, and on orchard grass in period 8. Group C was pastured on bluegrass during period 1, on the above legume mixture during periods 2, 4 and 6, on alfalfa, brome-grass and ladino clover in periods 3, 5 and 7, and on alfalfa-timothy in period 8. No. of cows per group was 6.

layer again is washed once with water, care being exercised to prevent the formation of an emulsion. Twenty-five ml. of a previously prepared saturated KOH-methyl alcohol solution then is added and the funnel shaken vigorously. The bottom layer is drawn off and then the process is repeated until this layer is colorless. The yellow top layer is washed three times with water and filtered through Na_2SO_4 (anhydrous) into a 100-ml. vol. flask. This crystal clear yellow solution, which contains the carotenoid pigments, then can be read directly or after suitable dilutions in a photoelectric colorimeter using a $440\text{m}\mu$ filter.

RESULTS AND DISCUSSION

The data showing the milk carotenoids and vitamin A during barn feeding prior to pasture and at each pasture period throughout the pasture season appear in fig. 1 (1945), fig. 2 (1946) and fig. 3 (1947). Milk carotenoids increased rapidly following the beginning of pasture consumption, usually reaching a peak in early June regardless of the kind of pasture eaten. In 1945 the milk carotenoids leveled off during most of May after a small initial rise. This was due to a cold wet spring in which the pastures stopped growing, resulting in a low carotenoid level of the herbage. Milk vitamin A showed an initial rise and then

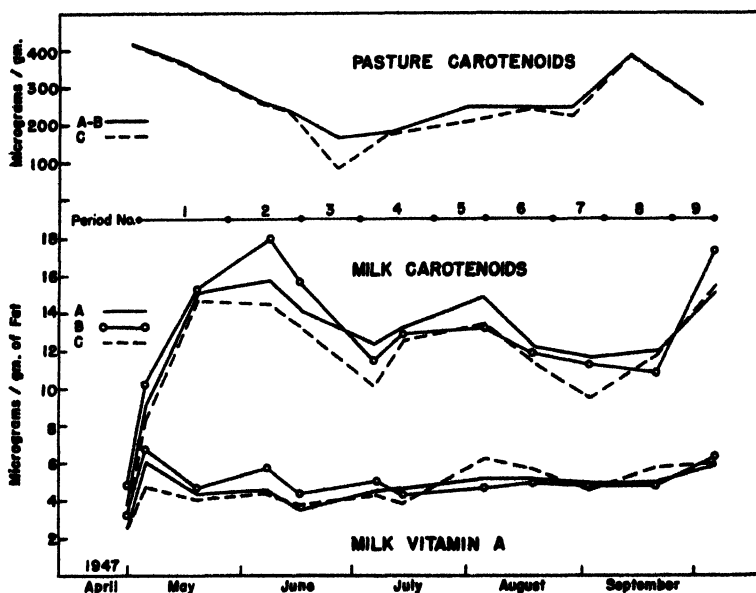


FIG. 3. The relation of the carotenoids in the pasture to the carotenoid and vitamin A content of the milk, 1947. Groups A and B were pastured on bluegrass during periods 1, 3, 5, 7 and 9, and on a legume mixture consisting of alfalfa, brome grass, ladino clover, and timothy in periods 2, 4, 6 and 8. Group C was pastured on bluegrass during periods 1 and 9, on the above legume mixture in periods 2, 4, 6 and 8, and on a legume mixture of alfalfa, brome grass, and ladino clover in periods 3, 5 and 7. No. of cows per group was 7.

tended to level off. Changes in the carotenoid content of the pasture herbage were not reflected as markedly in the milk vitamin A as in the milk carotenoids.

It is difficult to determine the level of pasture carotenoids above which no further increase in milk carotenoids and vitamin A is elicited. However, it appears from the data presented that the carotenoids in the milk follow the fluctuations in the carotenoids of the pasture much more closely when the pasture carotenoids fall below about 250 γ per g. than at levels above this value.

That the vitamin A level in the milk is subject to less fluctuation due to the intake of carotenoids from the forage is attributed to the influence of the liver

stores and also to the influence of the conversion of carotene to vitamin A. It is of interest in this connection that on numerous occasions the milk vitamin A was observed to increase at the same time the milk carotenoids decreased. This phenomenon has been observed before (2, 3, 10) in studies on blood carotenoid and vitamin A relationships. The appearance of this "inverse" relationship of vitamin A and carotenoids in milk is likely a reflection of similar changes in the blood.

An examination of the milk production curves revealed no indication that the milk carotenoids were influenced markedly by the stage of lactation.

No marked beneficial effect on milk carotenoids or vitamin A levels was attributed to the feeding of supplemental hay in this experiment. This does not mean that under conditions such as extreme drought or sparse grazing the feeding of good quality hay or silage would not increase the vitamin A and carotenoids in the milk.

These data serve to illustrate the fact that, so far as a source of vitamin A activity is concerned, good bluegrass pastures can be equally as good as legume mixtures. This is true provided there is sufficient rainfall and the pastures are managed so as to keep the bluegrass growing rapidly. The superior ability of the legumes to withstand periods of drought causes them to be better sources of carotene than bluegrass under such conditions.

SUMMARY

Fluctuations in the vitamin A and carotenoid content of summer milk produced by three groups of cows were related to the fluctuations in the carotenoid content of the pasture herbage consumed during three pasture seasons. The three groups included cows pastured on bluegrass, one group with and one without supplemental hay, and another on legume mixtures, without supplemental hay.

The data illustrate the following: (a) A closer relation exists between pasture carotenoids and milk carotenoids than between pasture carotenoids and milk vitamin A. (b) After the initial rise following the beginning of the pasture season, the fluctuations in milk carotenoids follow the changes in pasture carotenoids more closely when the pasture carotenoids level is below about 250 γ per g. than when it is higher, indicating a maximum response at this level. (c) Permanent bluegrass pasture, under weather and management conditions which favor rapid growth, often is equally as good a source of carotenoids as is a pasture consisting of legume mixtures. The superiority of legumes is evident during periods of drought.

REFERENCES

- (1) BOYER, P. D., SPITZER, R., JENSEN, C., AND PHILLIPS, P. H. Determination of Vitamin A and Carotene in Milk. A Rapid Extraction Procedure. *Ind. Eng. Chem., Anal. Ed.*, 16: 101-102. 1944.
- (2) BRAUN, W. Studies on the Carotenoid and Vitamin A Levels in Cattle. I. Seasonal Changes of the Carotenoid and Vitamin A Levels and the Normal Carotenoid-Vitamin A Ratio of the Blood. *J. Nutr.*, 29: 61-71. 1945.

- (3) HIBBS, J. W., AND POUNDEN, W. D. The Influence of the Ration and Early Rumen Development on the Changes in the Plasma Carotenoids, Vitamin A and Ascorbic Acid of Young Dairy Calves. *J. Dairy Sci.*, 31: 1055-1061. 1948.
- (4) HODGSON, R. E., KNOTT, J. C., MURER, H. K., AND GRAVES, R. R. The Relation of Color and Carotene Content of Roughage in the Dairy Ration to the Color, Carotene Content and Vitamin A Activity of Butterfat. *J. Agr. Research*, 57: 513-528. 1938.
- (5) KOEHN, C. J. Vitamin A Activity of Milk as Related to Pasture and Feeding Practices in Alabama. *J. Dairy Sci.*, 26: 673-681. 1943.
- (6) KRAUSS, W. E., SKINNER, LOUISE, HIBBS, J. W., ARMSTRONG, T. V., AND SLATTER, W. L. Vitamin A Potency of Ohio Butter. *Ohio Expt. Sta. Bimonthly Bull.*, 30: 157-163. 1945.
- (7) LORD, J. W. Seasonal Variation of Carotene and Vitamin A in Butterfat and Serum. *Biochem. J.*, 39: 372-374. 1945.
- (8) MITCHELL, J. H., AND WISE, G. H. The Comparative Effects of Continual and Rotational Systems of Grazing on the Carotene Content of Permanent Pasture Herbage and of the Milk Produced Therefrom. *J. Dairy Sci.*, 27: 189-196. 1944.
- (9) SARKAR, B. C. RAY. Effect of Season, Breed and Species of Ruminants on the Vitamin A Potency of Butterfat. *J. Dairy Sci.*, 31: 165-172. 1948.
- (10) SUTTON, T. S., AND SOLDNER, P. A. Seasonal Variations in the Blood Plasma Carotene and Vitamin A of Adult Dairy Cattle. *J. Dairy Sci.*, 28: 859-867. 1945.

INFLUENCE OF PENICILLIN AND OTHER ANTIBIOTICS ON LACTIC STREPTOCOCCI IN STARTER CULTURES USED IN CHEDDAR CHEESEMAKING¹

H. KATZNELSON² AND E. G. HOOD³

Central Experimental Farm, Ottawa, Canada

In a recent note, Whitehead (17) warned cheese factory patrons that milk from cows infected with bovine mastitis and under treatment with penicillin should not be sent to a cheese factory during the period of treatment and for at least 1 day thereafter, since penicillin has an effect upon starter action. Hunter (6) in a more detailed study of this problem found that *Streptococcus cremoris* strains were inhibited markedly by doses of 0.1 unit per ml., whereas *Streptococcus lactis* strains were more tolerant, being inhibited by 0.25 to 0.3 unit per ml. of milk (as measured by lactic acid production). Essentially, similar results were reported by Hood and Katznelson (5, 7) using a single and a mixed strain starter culture. As little as 0.05 unit per ml. of Na penicillin G gave marked inhibition of acid production. Preliminary tests also indicated that penicillinase inhibited the action of penicillin in milk and permitted acid production. The work reported below represents an extension of these earlier studies with different starters, different antibiotics, individual strains of lactic streptococci and penicillin-resistant starter cultures.

EXPERIMENTAL PROCEDURE AND RESULTS

The procedure employed in testing the effect of penicillin on acid production by starter cultures has been described in detail elsewhere (5). Pasteurized milk was inoculated with coagulated skim milk cultures of the starters at the rate of 3 per cent, dispensed in 100 ml. amounts in bottles and known amounts of penicillin or of other antibiotics added to each bottle. These were incubated in a water bath at 37° C., 9 ml. amounts being removed hourly and titrated with 0.1 N NaOH. Six starters in all were tested, two being single strain cultures.

The data in table 1 show typical results with four mixed strain starters. Complete inhibition of acid production by all cultures was obtained with 0.5 unit, strong inhibition with 0.1 unit and moderate with 0.05 unit per ml. milk. Similar results were obtained with the single strain cultures.

Five other antibiotics were tested in a similar manner and compared with penicillin; the results are summarized in table 2. Penicillin is the most active substance, with aureomycin and subtilin equal in regard to dilutions giving complete inhibition of growth. However, both penicillin and subtilin cause

Received for publication July 14, 1949.

¹ Contribution no. 289 (Journal Series) from Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

² Bacteriologist.

³ Chief, Dairy Research.

TABLE 1

Effect of penicillin on acid production in milk by four mixed strain starter cultures

Starter	Penicillin units/ml.	Titratable acidity after incubation for:				
		1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
KAS		(%)	(%)	(%)	(%)	(%)
	0.50	0.20	0.20	0.20	0.20	0.20
	0.10	0.20	0.24	0.32	0.35	0.36
	0.05	0.20	0.25	0.35	0.42	0.45
	0.01	0.20	0.24	0.35	0.46	0.57
	0.005	0.20	0.24	0.35	0.45	0.55
CENT	0	0.20	0.25	0.36	0.47	0.60
	0.50	0.19	0.20	0.20	0.20	0.20
	0.10	0.20	0.24	0.28	0.30	0.31
	0.05	0.20	0.24	0.30	0.37	0.40
	0.01	0.20	0.24	0.31	0.42	0.51
	0.005	0.20	0.24	0.32	0.44	0.55
OAC-H	0	0.20	0.24	0.32	0.44	0.55
	0.50	0.19	0.20	0.20	0.20	0.20
	0.10	0.20	0.25	0.31	0.37	0.39
	0.05	0.20	0.25	0.33	0.46	0.50
	0.01	0.20	0.25	0.33	0.49	0.61
	0.005	0.20	0.25	0.33	0.49	0.61
M	0	0.20	0.25	0.33	0.49	0.61
	0.50	0.20	0.20	0.20	0.20	0.20
	0.10	0.20	0.25	0.30	0.37	0.37
	0.05	0.20	0.26	0.33	0.48	0.53
	0.01	0.20	0.26	0.33	0.54	0.62
	0.005	0.20	0.26	0.33	0.54	0.63
	0	0.25	0.26	0.33	0.54	0.63

inhibition over a wider range of dilutions than do the remainder of the antibiotics. Considerably more chloromycetin and bacitracin are needed to give complete inhibition of starter action; the former appears to be the least effective of all the antibiotics studied, giving no inhibition at a dilution of 1:5,000,000.

Forty-five strains of lactic streptococci were isolated from a starter culture and their sensitivity to penicillin tested in Difco whey broth and in skim milk. Turbidity was used as the index of growth in the former medium and coagulation and/or reduction of pH of the milk (as measured with chlor-phenol red indicator) in the latter medium. Identified cultures of *S. cremoris*,⁴ and *Leuconostoc citrovorum*⁵ were included in this series. The cultures were less

TABLE 2

Influence of six antibiotics on acid production in milk by a mixed strain starter culture

Antibiotic	Reciprocal of dilution necessary for:	
	Complete inhibition	No inhibition
Penicillin	3,300,000	166,000,000
Streptomycin	500,000	20,000,000
Aureomycin	1,000,000	20,000,000
Chloromycetin	100,000	5,000,000
Subtilin	1,000,000	100,000,000
Bacitracin	100,000	20,000,000

⁴ Supplied by C. E. Parmelee, Iowa State College.⁵ American Type Culture Collection no. 8082.

TABLE 3
Inhibition of lactic streptococci in whey broth and skim milk by penicillin

Penicillin units/ml.	Strains isolated from starters ^a			
	Whey broth		Skim milk	
	Partial	Complete	Partial	Complete
0.4		45		45
0.2		45	7	38
0.1	5	40	35	8
0.05	14	27	23	0
0.025	18	11	12	0
0.0125	21	3	7	0
0.00625	9	1	4	0
Identified cultures ^b				
<i>Streptococcus cremoris</i> HI-1	0.05	0.2	0.1	0.4
<i>Streptococcus cremoris</i> ML1	0.05	0.2	0.1	0.4
<i>Leuconostoc citrovorum</i> 531	0.4	1.6	0.8	> 1.6

^a Number of strains out of 45.

^b Units penicillin/ml.

sensitive to penicillin in milk than in whey broth (table 3). In the former, all strains required from 0.2 to 0.4 unit penicillin per ml. for complete inhibition; in the latter, most of the cultures were completely inhibited by 0.05 to 0.1 unit, as well, and several even with 0.00625 to 0.0125 unit. The identified cultures fell in the more resistant group of strains both in whey broth and in skim milk; *L. citrovorum* was the most resistant of all the cultures tested.

As has been reported previously (5, 7) the effect of penicillin on starter culture activity was overcome by the addition of penicillinase at the rate of 0.02 mg. concentrate per 100 ml. of milk. The earlier work was repeated and the data condensed as shown in table 4. The enzyme permitted almost normal acid production at a concentration of penicillin (0.5 unit per ml.) which otherwise stopped fermentation completely. Fifty per cent of the total acid produced in the control at 5 hr. was produced with 1.0 unit penicillin per ml. in presence of penicillinase. Cysteine in amounts ranging from 1.0 to 10.0 mg. per 100 ml. of milk did not inactivate penicillin; in fact, the amino acid was somewhat stimulatory in the lower concentrations.

TABLE 4
Effect of penicillinase on acid production by a mixed strain starter culture in milk containing penicillin

Penicillin units/ml.	% titratable acidity after 5 hr.	
	No penicillinase	With penicillinase
5.0	0.20	0.20
1.0	0.20	0.39
0.5	0.20	0.45
0.1	0.34	0.53
0.05	0.42	0.58
0.01	0.51	0.58
0.005	0.54	0.58
0	0.57	0.58

TABLE 5

Resistance of penicillin in milk to pasteurization, as determined by acid production by mixed strain starter culture

Penicillin units/ml.	% titratable acidity after 5 hr.	
	Unpasteurized	Pasteurized
5.0	0.18	0.18
1.0	0.18	0.18
0.5	0.18	0.18
0.1	0.40	0.39
0.05	0.44	0.43
0.01	0.59	0.60
0.005	0.58	0.59
0.0	0.59	0.61

Pasteurization of milk containing penicillin was considered as another means of overcoming the inhibitory effect. Hunter (6) reported that heating milk for 30 min. at 145° F. resulted in very little loss in potency of added penicillin; steaming for 1 hr. resulted in approximately 50 per cent loss. In the present study increasing amounts of penicillin were added to raw milk, one set of bottles was pasteurized for 30 min. at 145° F. and another remained as control. All bottles then were inoculated at the rate of 3 per cent, incubated and sampled as indicated earlier. Results in table 5 show no effect whatever on penicillin activity due to pasteurization and thus corroborate Hunter's findings (6).

Another means of obviating the effect of penicillin is by use of resistant cultures. It is well recognized that bacteria may be adapted to tolerate appreciable amounts of various inhibitory agents by being transferred in increasing amounts of these substances (15). Accordingly, this was done with six

TABLE 6

Resistance of an "adapted" culture to penicillin before and after subculture in penicillin-free milk

Penicillin units/ml.	Titratable acidity developed by:		
	Adapted starter	Adapted starter after 20 transfers in penicillin- free milk	Unadapted control starter
	(%)	(%)	(%)
0	0.71	0.82	0.89
0.2	0.70	0.81	0.50
0.4	0.69	0.81	0.23
0.6	0.69	0.83	0.21
0.8	0.69	0.79	0.21
1.0	0.68	0.78	0.21
1.2	0.67	0.83	0.20
1.4	0.60	0.81	0.21
1.6	0.65	0.79	0.20
1.8	0.60	0.77	0.21
2.0	0.66	0.73	0.20
2.2	0.62	0.73	0.21
2.4	0.60	0.65	0.21
2.6	0.61	0.65	0.20
2.8	0.61	0.64	0.20
3.0	0.59	0.62	0.21

starter cultures in sterile skim milk containing penicillin. Coagulation of the milk after overnight incubation at 21° C. was taken as the index of satisfactory development of the starter. One of these starters retained its ability to coagulate milk after daily transfers for 4 mo., the others did not. During this period the culture gradually was adapted to increasing amounts of penicillin, completely coagulating milk with 2.1 units per ml. Since it has been shown that such "trained" cultures can lose their resistance after subculture in absence of the antibiotic (15), the above culture when able to tolerate 1.8 units per ml. was transferred to penicillin-free milk. After six and twenty passages its sensitivity to increasing amounts of penicillin was determined. There was no change whatever in its resistance to penicillin after six transfers in penicillin-free milk and as the results in table 6 clearly show, its resistance remained substantially unchanged even after 20 transfers. In fact, it coagulated milk completely even in the presence of 3.0 units penicillin per ml. of milk, although its acid-producing capacity was appreciably lower at this level. The adapted starter also coagulated milk in the presence of 3.0 units penicillin per ml. and similarly showed a decrease in acid-producing capacity. Its vitality in general was decreasing as compared with the other two starters. The unadapted culture was completely inhibited by 0.4 unit and produced less than 50 per cent of the total acidity with 0.2 unit of penicillin per ml. of milk.

DISCUSSION

Although the results presented show considerable variation in penicillin sensitivity among the 45 strains isolated from starter cultures, it is clear that these strains are all completely or partially inhibited by penicillin levels which completely or partially prevent acid formation by the starters themselves. Considerable variation in tolerance exists among the streptococci (2, 14), the enterococci being among the most resistant; according to the results obtained by Hunter (6) and to the data presented above, the lactic streptococci are among the most sensitive.

Other antibiotics such as tyrothricin, bacitracin and streptomycin have also been used for control of bovine mastitis (1, 4). From the point of view of the problem of carry-over of antibiotics into cheese milk the most desirable agent would be one which controlled bovine mastitis and to which the lactic streptococci were most tolerant. Chloromycetin appears to satisfy the latter criterion, being the least potent of the antibiotics tested. Therefore, it remains to be seen if it will be effective in the treatment of bovine mastitis. Little, if any, information is available on the effect of chloromycetin on lactic streptococci; however, 0.63 γ per ml. will cause a 50 per cent reduction of growth of *Streptococcus pyogenes* (12).

A very natural question may arise as to the amount of penicillin which might find its way into milk and thus cause trouble. Where only a few animals in an area are under treatment, the dilution of the antibiotic in the pooled milk supply would obviate the difficulty. However, as Hunter (6) and the authors (5, 7) have pointed out, where large numbers of animals in an area are being

treated simultaneously with penicillin the matter of carry-over into cheese milk might be of considerable concern to the cheese manufacturer. Various workers have reported on the concentrations of penicillin in milk from treated quarters, but the amounts which are present in the final, pooled cheese milk are much more difficult to estimate and will vary depending on the amount of penicillin infused, the number of quarters treated, the amount of milk produced by each animal, number of infected animals in a herd, number of animals milked and the volume of milk from treated animals in relation to the total volume of cheese milk. Weirether *et al.* (16) reported that milk contained 0.5 or more O.U. per ml. 24 hr. after infusion of 30,000 or more O.U. per quarter. An analysis of their data shows as much as 10.0 O.U. per ml. 24 hr. after infusion of 40,000 O.U. and about 40 O.U. at 12 hr. Schalm and Casselberry (10) reported that 12 hr. after infusion of 20,000 units per quarter there was an average of 4.95 units per ml. of foremilk in 14 quarters in six udders; with six quarters in three udders an average of 14.29 units per ml. was found. When eight dry quarters each were treated with 100,000 units, there were 5.5 to 29.0 units per ml. of milk 24 hr. afterwards in seven quarters and 0.4 units in one; after 48 hr. seven quarters had 0.7 to 4.7 units per ml. Packer's data (9) indicate an average of 27.8 units per ml. 12 hr. and 2.1 units per ml. 24 hr. after infusion and Hunter (6) arrives at a figure of 5 units per ml. as a conservative estimate after infusion of 25,000 units per quarter with indications of even higher values. Similar wide variations have been reported by other workers (8, 11, 13).

Hunter calculates that 90 gal. of milk with 5 units per ml. in 4,500 gal. (a five-vat factory supply per day) would provide the critical level of 0.1 unit per ml., causing appreciable reduction in acid production. This figure is almost identical with the critical level obtained in the present study causing about 50 per cent reduction of acid production (table 1). On the basis of Packer's data, calculations were made which indicate some of the complications involved and show that the problem can actually arise under certain circumstances.

	Penicillin concentrations	
	12 hr. after infusion (units/ml.)	24 hr. after infusion (units/ml.)
Milk from treated quarter	27.8	2.1
If 2 quarters treated, pooled milk of 4 quarters	14.0	1.0
If 1 of 5 cows treated, pooled milk	2.8	0.2
If 1 of 10 cows treated, pooled milk	1.4	0.1

It would appear from this hypothetical consideration that in areas where 10 per cent of the animals or more were under treatment, sufficient penicillin might be found in the milk to cause trouble in the cheese factory.

The most serious danger might occur in connection with preparation of starter cultures since milk from one patron usually is used; however, as Hunter points out, careful selection of milk to ensure its coming only from untreated cows would overcome this difficulty. Whitehead (17) suggested that milk obtained from cows during penicillin treatment and for 1 day thereafter be withheld from the cheese factory. This is the simplest means of avoiding trouble

but one which is not likely to appeal to cheese factory patrons. Furthermore, in the light of recent reports on the use of various vehicles, such as mineral oil, water and lanolin derivatives, propylene glycol, etc., designed to sustain penicillin levels in the mammary glands (3), Whitehead's suggestion may be impractical since by these means penicillin has been found in milk 72 hr. after infusion in amounts of 0.4 to 4 units per ml. The use of penicillin-resistant starter cultures would obviate the trouble and preclude loss of milk. Pasteurization is ineffective and addition of impure penicillinase concentrates to cheese milk may be objectionable because of expense, possible toxicity and pure food regulations.

SUMMARY

Complete inhibition of acid production by six starter cultures was obtained with 0.5 unit penicillin per ml. of milk, strong inhibition with 0.1 unit and moderate with 0.05 unit.

Penicillin was the most inhibitory of the six antibiotics tested with subtilin next; chloromycetin was the least potent.

All 45 strains of lactic streptococci isolated from starter cultures were completely inhibited by penicillin in skim milk in amounts ranging from 0.2 to 0.4 unit per ml. In whey broth most of the cultures were completely inhibited by 0.05 to 0.1 unit; several were sensitive to 0.00625 and 0.0125 unit. A culture of *Leuconostoc citrovorum* tested required 1.6 units for complete inhibition.

Penicillinase at the rate of 0.02 mg. per 100 ml. of milk permitted almost normal acid production by a starter at a concentration of penicillin (0.5 unit per ml.) which stopped fermentation completely and permitted 50 per cent acid production in the presence of 1.0 unit per ml.

Cysteine in amounts ranging from 1.0 to 10.0 mg. per 100 ml. milk was ineffective against penicillin, as was pasteurization.

A penicillin-resistant starter culture was developed which coagulated milk in the presence of 3 units penicillin per ml. and retained its resistance after 20 passages in absence of penicillin.

Calculations are included on the amounts of penicillin which may be found in milk used for cheesemaking.

REFERENCES

- (1) BENSON, D. V. Treatment of Bovine Mastitis with Tyrothricin, Bacitracin, Streptomycin, and Penicillin. J. Am. Vet. Med. Assoc., 111: 289-294. 1947.
- (2) BORNSTEIN, S. Action of Penicillin on Enterococci and other Streptococci. J. Bact., 39: 383-387. 1940.
- (3) FOLEY, E. J., STULTZ, A. W., LEE, S. W., AND BYRNE, J. V. Studies on Vehicles for Sustaining Penicillin Levels in the Bovine Mammary Glands. Am. J. Vet. Research, 10: 66-70. 1949.
- (4) HERRELL, W. E. *Penicillin and other Antibiotic Agents*. W. B. Saunders Co., Philadelphia and London. 1945.
- (5) HOOD, F. G., AND KATZNELSON, H. The Effect of Penicillin on the Acid-Producing Ability of Starters. Can. Dairy and Ice Cream J., 28: 32-33. 1949.
- (6) HUNTER, G. J. E. The Effect of Penicillin on Lactic Streptococci. J. Dairy Research, 16: 39-45. 1949.

- (7) KATZNELSON, H., AND HOOD, E. G. Penicillin in Relation to Acid Production in Milk by Starter Cultures used in Cheddar Cheesemaking. *Science*, 109: 490-491. 1949.
- (8) MURNANE, D. A Preliminary Report on the Treatment of Clinical and Subclinical Streptococcal and Staphylococcal Infections of the Bovine Udder with Penicillin. *Aust. Vet. J.*, 21: 89-90. 1945.
- (9) PACKER, R. A. Penicillin Therapy in Chronic Bovine Mastitis. II. Penicillin Levels in the Udder During Treatment. *Am. J. Vet. Research*, 9: 259-263. 1948.
- (10) SCHALM, O. W., AND CASSELBERRY, W. A. Treatment of *Streptococcus agalactiae* Infections with Intramammary Infusions of Penicillin. *J. Am. Vet. Med. Assoc.*, 109: 470-477. 1946.
- (11) SCHOFIELD, F. W. Penicillin in the Treatment of Bovine Mastitis. *Can. J. Comp. Med. Vet. Sci.*, 10: 63-70. 1946.
- (12) SMITH, R. M., JOSLYN, D. A., GRUHZIT, O. M., MCLEAN, I. W., PENNER, M. A., AND EHRLICH, J. Chloromycetin Biological Studies. *J. Bact.*, 55: 425-448. 1948.
- (13) STEVENSON, W. G. The Concentration of Penicillin in Fore Milk Following Intramammary Infusion for the Treatment of Mastitis. *Can. J. Comp. Med. Vet. Sci.*, 10: 82-83. 1946.
- (14) THORP, W. T. S., UHRIK, I. J., AND STRALEY, E. J. Concentrations of Penicillin in the Bovine Mammary Gland Following Infusion and Penicillin Tolerance of Certain Streptococci. *Am. J. Vet. Research*, 8: 157-165. 1947.
- (15) WAKSMAN, S. A. *Microbial Antagonisms and Antibiotic Substances*. 2nd ed. The Commonwealth Fund. New York. 1947.
- (16) WEIRETHER, F. J., JASPER, D. E., AND PETERSON, W. E. Effect of Infused Penicillin in the Bovine Mammary Gland. *Proc. Soc. Exptl. Biol. Med.*, 59: 282-286. 1945.
- (17) WHITEHEAD, H. R. Penicillin—The Answer to "Strep" Mastitis? *New Zealand Dairy Exporter*, 24 (1), July 1, 1948.

NUTRIENT COMPOSITION OF BANANA SKINS¹

J. G. ARCHIBALD

Animal Husbandry Dept., University of Massachusetts, Amherst

Recently, someone who makes a practice of feeding kitchen and garden refuse to family livestock sent in an inquiry as to the feeding value of such substances. For most of the items mentioned there is considerable information on record, but of the lowly banana skin little is known. The information found was in reports of some analyses made in England and Germany (1, 3), results of one digestion trial with sheep in Germany (2) and an account of a study of the pigments of the banana skin from this country (4), all made many years ago.

Therefore, it was decided to make a rather detailed analysis of the skins. The sample obtained for the work was representative of an entire bunch of the whole fruit. Care was taken in sampling to separate from the skins and discard any pieces of the pulp which either had been overlooked or had been purposely discarded as too ripe for human consumption.

Moisture and carotene were determined in separate portions of the freshly removed skins; the bulk of the sample then was dried rapidly at approximately 80° C. and ground for analysis. The accompanying table gives the values obtained² and, for comparison, such other data as are available.

TABLE 1
Composition of banana skins

	Our analyses	Honcamp <i>et al.</i> (2)	Leuschner (3)
	(%)	(%)	(%)
Natural moisture	83.8	88.0	70.0
Constituents of the dry matter			
Protein	6.1	7.7	9.7
Ether extract	8.7	8.1	13.7
Crude fiber	10.0	8.6	28.7
Nitrogen-free extract	63.1	65.1	34.7
Total sugar (as invert sugar)	22.0		
Total ash	12.1	10.5	13.0
Soluble ash	11.7		
Calcium	.35		
Magnesium	.23		
Sodium	trace		
Potassium	5.72		
Phosphorus	.32		
Sulfur	trace		
Chlorine	.64		
	(p.p.m.)		
Carotene	66.0		

Received for publication July 15, 1949.

¹ Contribution 730 of the Massachusetts Agricultural Experiment Station.

² Most of the analyses reported were made in the State Feed Control Laboratory under the supervision of J. W. Kuzmeski, Official Chemist in charge.

There are some rather surprising values here; worthy of special mention are the relatively large amounts of ether extract, sugar, carotene and soluble ash. The ether extract probably carries some indigestible waxy material such as cutin or suberin, but a good deal of this portion doubtless is the familiar banana oil (amyl acetate). The pleasing, characteristic odor of the dried and ground product strongly suggests this possibility. Any material that contains as much as 22 per cent of sugar in the dry matter should not be dismissed as worthless.

The level of carotene is much higher than that reported in the study already referred to (5)—11 p.p.m. in the fresh skins in contrast with about 2.5 p.p.m.; this difference may be due to greatly improved methods for carotene determination, since the work quoted was published in 1929. If the skins were processed in any way, probably a good deal of the carotene would be destroyed, but since most of them, if used at all, are offered to farm livestock soon after removal of the edible portion, they can constitute an additional and not inconsiderable source of vitamin A.

The unusual feature of the ash content is the high value for potassium; this, of course, is not significant nutritionally, but is of interest as perhaps indicating the need for liberal use of potash salts in the culture of the banana plant. In view of the role of potassium in starch formation and translocation it is not surprising to find high concentrations of this element in a fruit as predominantly carbohydrate in nature as the banana. Another investigator (1) reports a somewhat higher value for potassium than was found here (7.5 per cent of the dry substance) and the observation has been made that "the high figure for potassium common to [banana] peel and pulp has suggested the collection of banana waste, the ash of which would form a valuable fertilizer" (4). The difficulty in this connection is, of course, the accumulation of a worthwhile tonnage.

There also is the further possibility, subject to the same limitations, of drying and grinding the skins discarded in the manufacture of banana flour, banana flakes, etc. and marketing the product as a constituent of ready-mixed feeds for livestock. If economically feasible, such a disposition of the skins and any cull fruit³ would be much less wasteful than burning them to recover the ash. Although not in a class with the standard cereal grains and many of the feed concentrates, it has been shown (2) that banana skin meal has a nutrient value of approximately 56 per cent, which is comparable with a very good grade of hay, and somewhat above much of the hay ordinarily fed to livestock and certain industrial by-products now being used as ingredients of some ready-mixed feeds.

³ The nutritive value of the product would be increased in direct proportion to the amount of cull whole fruit included.

REFERENCES

- (1) ELLIS, R. H. Note on the Presence of Potash in Banana Skins. *J. Soc. Chem. Ind.*, **35**: 521. 1916.
- (2) HONCAMP, F., GOETTSCH, H., GSCHWENDNER, B. *et al.* Untersuchungen über die Zusammensetzung und Verdaulichkeit einiger landwirtschaftlicher Produkte aus Deutschlands afrikanischen Kolonien. *Landw. Vers. Sta.*, **77**: 305-330. 1912.
- (3) LEUSCHER. *Zeit. öffentl. Chem.*, **8**: 125. 1902. Cited in Thorpe's *Dictionary of Applied Chemistry*. Revised and enlarged ed., **1**: 534. 1921. (Original paper not available.)
- (4) THORPE, J. F. *Dictionary of Applied Chemistry*. 4th ed., **1**: 619.
- (5) VONLOESECKE, H. Quantitative Changes in the Chloroplast Pigments in the Peel of Bananas During Ripening. *J. Amer. Chem. Soc.*, **51**: 2439. 1929.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXII

DECEMBER, 1949

NUMBER 12

A NEW LYOPHILIZING APPARATUS

J. W. STULL¹, AND E. O. HERREID

Department of Food Technology, University of Illinois, Urbana

In connection with a research project dealing with some heat labile fractions of milk, it was desired to build a lyophilizing apparatus. The lyophilizing process (drying from the frozen state) was described in detail first by Shackell (10) in 1909. Five years later, Rogers (8) described the design, construction and application of another lyophilizing apparatus. His classic study of the preparation of dried cultures of microorganisms apparently did not arouse much interest until 1935, when two groups of workers (4, 5) published descriptions of lyophilizing apparatus and discussed their application to the drying of biological substances, including cultures of microorganisms. A summary of the theory of the lyophilizing process is given by Bradish (1) and Bradish *et al.* (2). A review of the recent literature dealing with the construction of lyophilizing apparatus revealed a wide variation in design, size, simplicity and efficiency of operation (1, 2, 3, 6, 7, 9, 11). Without going into detail concerning the theory of the lyophilizing process, it may be said that the construction of the equipment described herein, provides for the optimum conditions of the lyophilizing operation, namely, (a) the maintenance of a high vacuum, (b) short, direct path from the surface of the material to a condenser which is maintained at the temperature of dry ice, and (c) drying from a thin layer of frozen material.

Construction of apparatus. Fig. 1 shows the lyophilizing apparatus. The outer member of a 71/60 standard taper ground glass Pyrex joint was fitted so as to replace the neck of a 3 l. round bottom flask (A). At a point 2.0 cm. above the junction of the neck with the body of the flask, the outer member of a 29/42 standard taper ground glass Pyrex joint was sealed as a side arm into the neck at an angle of 45°.

The condenser was constructed from a glass tube (o.d. 3.0 cm.) which was ring-sealed at the top of the inner member of the 71/60 ground glass joint. The tube was sealed off at the bottom such that there was a clearance of 3.0 cm. between it and the bottom of the flask when the inner member was placed into position.

Four complete units² were constructed to be connected to the manifold (B) through which the vacuum was applied by means of a mercury diffusion pump.

Received for publication July 11, 1949.

¹ Now at the University of Arizona, Tucson.

² Two units were constructed from 3 l. round bottom flasks and two from 5 l. flasks.

The manifold was constructed from a 500 ml. round bottom flask. The four inner members of the 29/42 standard taper joints were sealed into the bottom of the flask at an angle of 135° with the neck of the flask. A 2.0 mm. two-way stopcock was sealed into the bottom of the manifold to be used in releasing the vacuum. The vacuum was applied at the top of the manifold.



FIG. 1. Assembled apparatus with the vacuum system attached. A—lyophilizing units. B—manifold. C—liquid air trap. D—mercury diffusion pump. E—backing pump.

Operation of the apparatus. The operation of the apparatus is as follows:

- (a) Pour the material to be dried into the flask.
- (b) Immerse the flask and contents in an acetone-dry ice bath and freeze the material on the inside surface of the flask in a thin, uniform layer by swirling.
- (c) Place the condenser into position and connect the assembly to the manifold.
- (d) Apply the vacuum.
- (e) Place acetone-dry ice coolant in the condenser tube at a level even with or above the side arm of the flask.
- (f) Place a fan³ in a position which will direct a current of air against the flasks.
- (g) Drying is complete when the flasks reach room temperature. Release the vacuum, disconnect each assembled unit from the manifold and remove the inner member which holds the moisture in the form of ice.

Operating characteristics. Typical operating results are found in table 1.

³ A 16-in. ventilating fan was placed within 6 in. of the flasks and operated at full speed.

TABLE 1
Typical results obtained in the operation of the lyophilizing apparatus

Product	Trial no.		
	1	2	3
	Buttermilk	Condensed washed cream buttermilk	Condensed washed cream buttermilk
Wt. of product (g.)	230	230	275
Total solids (%) ^a	11.5	10.1	10.1
Wt. of powder (g.)	27.3	23.2	27.5
Moisture (%)	2.9	3.5	3.7
Time of operation (hr.)	5	3	3
Room temp. (° C.)	22-23	28-29	28-29
Size of flask (l.)	3	3	5
Mean rate of moisture removal (g./hr.)	40.4	69.7	82.2
Vacuum applied (mm. Hg.) ^b	0.1	0.1	0.1
Wt. of water removed (g.)	201.9	206.0	246.5

^a Mojonner Method.

^b Determined by vacuum arc tester.

In regard to the rate and amount of moisture removal, three points should be emphasized. First, it is important to secure the vacuum reported not only to maintain the maximum rate but also to derive the indicated capacity of the apparatus. The latter factor results from the fact that the capacity of the apparatus is limited by the maximum thickness of the ice formation which can clear the neck of the flask when the condenser is removed. It has been found, therefore, that as the vacuum applied is increased the structure of the ice changes from fine, loosely formed crystals to a dense, compact mass at a pressure of less than 0.1 mm.

The explanation of the variation in the rates of moisture removal in trials 1, 2 and 3 (table 1) is probably found in the second and third points to be considered, namely, the room temperature and the size of the unit. An increase in room temperature of 6° C. resulted in an increase in rate of moisture removal of more than 50 per cent (trial 1 vs trial 2). Finally, through an increase in the size of flask from 3 to 5 l. (trial 2 vs trial 3), the rate of moisture removal was raised approximately 20 per cent.

CONCLUSIONS

A lyophilizing apparatus is described in which the design, simplicity, and efficiency represent improvements in the types of apparatus available for small scale operation.

REFERENCES

- (1) BRADISH, C. J. Freeze Drying. *Chem. Products*, 10 (9-10): 60-66. 1947.
- (2) BRADISH, C. J., BRAIN, C. M., AND MCFARLAND, A. S. Vacuum Sublimation of Ice in Bulk. *Nature*, 159 (4027): 28-29. 1947.

- (3) COHN, E. J., STRONG, L. E., HUGHES, W. L., MULFORD, D. J., ASHWORTH, J. N., AND TAYLOR, H. L. Preparation and Properties of Serum and Plasma Proteins. IV. A System for the Separation into Fractions of the Protein and Lypoprotein Components of Biological Tissues and Fluids. *Am. Chem. Soc.*, 68 (3): 459-475. 1946.
- (4) ELSER, W. J., THOMAS, R. A., AND STEFFEN, G. I. The Dessication of Sera and Other Biological Products (Including Microorganisms) in the Frozen State with the Preservation of the Original Qualities of Products so Treated. *J. Immunology*, 29 (5): 389-426. 1935.
- (5) FLOSDORF, E. W., AND MUDD, S. Procedure and Apparatus for Preservation in "Lyophile" form of Serum and other Biological Substances. *J. Immunology*, 29 (5): 389-426. 1935.
- (6) FRIEDGOOD, H. B., HAAGEN-SMIT, A. J., GAEST, J. B., AND STEINTZ, L. The Concentration and Preservation of Urinary Substances by Lyophilization. *Science*, 105 (2717): 99-100. 1947.
- (7) JACOBS, H. R. An Improved Lyophilizing Apparatus for Small-scale Operations. *Quart. Bull. Northwestern Univ. Med. School*, 21: 156. 1947.
- (8) ROGERS, L. A. The Preparation of Dried Cultures. *J. Infectious Diseases*, 14 (1): 100-124. 1914.
- (9) SEEGER, W. H. An Arrangement for Drying Proteins from the Frozen State. *Science*, 101 (2620): 284. 1945.
- (10) SHACKELL, L. F. An Improved Method of Dessication with Some Applications to Biological Problems. *Am. Physiol.*, 24 (3): 325-341. 1909.
- (11) SNYDER, W. E. A Study of the Fat Emulsion in Natural and Artificial Creams. Ph.D. Thesis, Univ. of Wis. 1948.

THE EFFECT OF INTERRUPTION OF MILKING ON THE CAROTENE AND VITAMIN A AND PROXIMATE COMPOSITION OF MILK AND ON THE CALCIUM CONTENT OF BLOOD SERUM¹

D. N. MERCER, H. D. EATON, R. E. JOHNSON, A. A. SPIELMAN,
W. N. PLASTRIDGE, L. D. MATTERSON AND L. NEZVESKY

*Animal Industries Department and Animal Diseases Department, Storrs Agricultural
Experiment Station, Storrs, Connecticut*

The interruption of milking has been found by several investigators (1, 3, 4, 8, 12, 13, 14, 16) to result in a marked alteration of the proximate composition of milk. In general, the amount of milk and the per cent lactose were found to have decreased, the per cent protein and ash to have increased, and the per cent fat to be affected inconsistently. During interruption of milking, an increase in the level of lactose in both blood and urine has been reported (1, 12). Although no reports on the effect of interruption of milking on blood serum calcium were found in the literature, blood serum calcium has been observed (10) to increase the third or fourth day postpartum in cows not milked following parturition. The objectives of this study were to determine the effect of interruption of milking for a 10-day period on (a) the carotene, vitamin A, and proximate composition of milk, and (b) the calcium level of blood serum.

EXPERIMENTAL

Animals. A total of 18 cows of the Ayrshire, Guernsey, Holstein and Jersey breeds were used in this experiment during the period March, 1948, through April, 1949. Twice daily milking was interrupted for a 10-day period in 12 of these cows; that is, no milk was removed from the udders of these cows during the 10-day period. The six remaining cows served as controls and were milked twice daily for the entire experimental period. The average number of lactations and the average number days milked postpartum were 1.8 ± 0.9 and 175.7 ± 26.6 , respectively, for those cows in which milking was interrupted and 4.0 ± 1.9 and 104.2 ± 50.2 for the controls.

For 4 wk. prior to the interruption of milking and for the experimental period, all cows received roughage on the basis of liveweight. This consisted per 100 lb. of liveweight of 1 lb. of U. S. No. 2 alfalfa hay and 3 lb. of well-matured corn silage. A grain mixture consisting largely of cereal grains and containing 13.5 per cent crude protein was fed according to milk production. To those cows in which milking was interrupted, no grain was fed during the first 8 days of the interrupted milking period. The hay, silage and grain contained on an average 17.71, 1.89, and 0.16 mg. of carotene per lb., respectively, as de-

Received for publication July 14, 1949.

¹ This work was supported in part by the Big-Y-Foundation, Norwich, Conn. and Chas. M. Cox Co., Boston, Mass. It is part of a thesis presented by D. N. Mercer to the Graduate School of the University of Connecticut in partial fulfillment of the requirements for the degree of Master of Science.

terminated by the method of Moore and Ely (9) as modified by Nelson *et al.* (11).

Samples. Representative samples from the six milkings immediately prior to the 10-day interruption period and from the first six milkings following interruption were obtained from 12 of the cows. Similar samples plus samples during the 10-day period when the treated cows were not milked were obtained from two of the control cows. Milk samples were chilled in the dark at 4° C. and analyses were completed in most instances within 6 days. When analyses could not be completed within 6 days, the samples were quick-frozen and held at -18° C. until analysed.

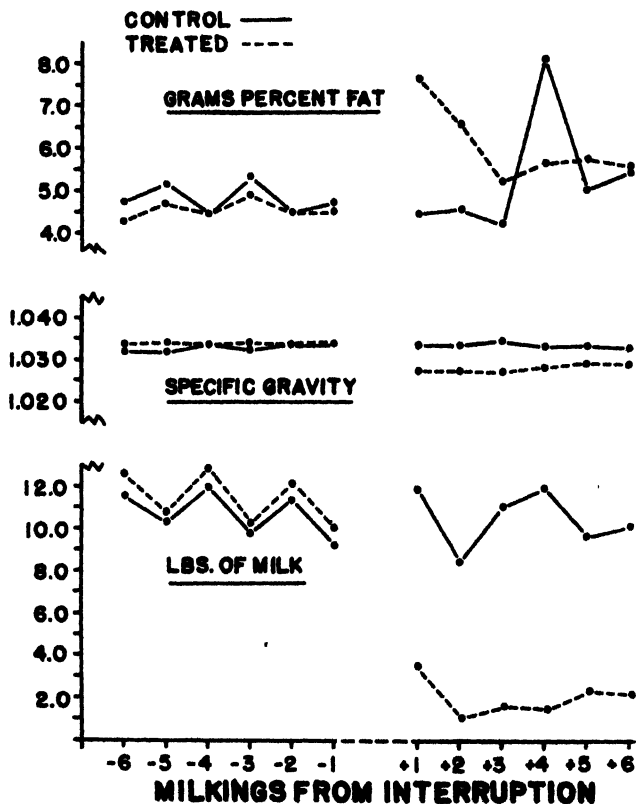


FIG. 1. The effect of interruption of milking on the lb. of milk, specific gravity and per cent fat.

Venous blood samples were obtained daily between 9 and 11 a.m. from four of the cows in which milking was interrupted and from four cows in which milking was not interrupted. The blood was allowed to clot at 4° C., centrifuged within 8 hr., and analyses completed for serum calcium within 72 hr. after collection.

Analyses. The methods used in the analyses of the milk samples were similar to those previously reported (5). Blood serum calcium was determined accord-

TABLE 1
The effect of interruption of milking on the level of calcium in blood serum

Exp. No.	Days															
	Before interruption			During interruption							After interruption					
	3	2	1	1	2	3	5	7	9	10	1	2	3	4	5	7
(mg. % serum calcium)																
Treated																
1	10.45	10.39	11.11	10.20	11.39	11.23	12.08	11.26	10.64	11.12	10.43	10.40	10.68	10.23	10.45	10.09
2	12.66	11.02	11.28	11.26	13.23	12.77	12.17	10.81	11.53	12.09	10.58	10.98	10.54	10.86	10.74	10.67
3	10.19	10.39	11.20	11.22	12.64	11.08	11.27	11.13	11.00	11.62	10.39	10.82	10.76	10.05	10.21	10.84
4	10.73	10.44	11.42	10.78	10.88	12.36	12.07	10.87	11.39	10.79	10.36	10.25	10.46	10.28	10.38	10.39
\bar{X}	11.01	10.56	11.22	10.87	12.04	11.86	11.90	11.02	11.14	11.41	10.44	10.61	10.61	10.36	10.45	10.70
Controls																
5	10.47	11.23	10.31	10.35	10.94 ^a	11.55	12.30	11.18	10.97	11.38	10.06	10.34	10.39	10.63	10.63	10.50
6	10.18	10.30	10.36	10.41	9.73 ^a	10.42	10.06	9.46	10.41	9.67	10.51	10.55	10.95	10.43	10.59	10.59
7	10.39	10.51	10.73	10.69	10.02 ^a	10.35	9.94	10.87	10.46	9.86	9.59	10.47	10.70	10.46	10.62	10.71
8	10.90	10.42	10.55	10.36	10.10 ^a	10.44	9.87	10.51	10.26	11.21	10.73	11.59	10.45	10.60	11.04	11.37
\bar{X}	10.49	10.62	10.49	10.45	10.20	10.69	10.54	10.51	10.53	10.53	10.22	10.74	10.62	10.53	10.72	10.79

^a Observation calculated according to missing plot technique (15).

ing to the method of Clark and Collip (2). Standard statistical procedures for the analysis of variance (15) were used to test for differences between treatments.

RESULTS

Data for the mean carotene and vitamin A and proximate composition of the milk for the six milkings prior to interruption and for the six milkings after interruption are given in figs. 1, 2, 3 and 4. The calcium levels in the blood serum of individual cows prior to, during, and after the interruption of milking are contained in table 1. Interruption of milking resulted in a decrease in the per cent lactose, and increases in the per cent carotene, vitamin A, protein, fat, and ash. With the exception of carotene and vitamin A, the total amount of these nutrients secreted was less after interruption than before interruption. The levels of blood serum calcium in those cows in which milking was interrupted were found to increase during the interruption period.

The average amount of milk and specific gravity (fig. 1) of the milk of those cows in which milking was interrupted was significantly less after interruption than prior to interruption. An analysis of the differences of the average values before interruption from the average values after interruption gave highly significant differences ($P < 0.001$) between treatments. Further analyses between average values for the six milkings prior to interruption and similar average values after interruption within treatments showed a similar statistical difference for the group in which milking was interrupted, but no statistical difference was found for the control group.

The average carotene and vitamin A content per 100 ml. of milk or calculated to per gram of fat (fig. 2 and 3) increased markedly after interruption of milking. The differences in the average values before interruption from those after interruption were statistically significant between treatments for vitamin A ($P < 0.01$ for per 100 ml. of milk and $P < 0.05$ for per g. of fat) but not for carotene. Within the group in which milking was interrupted, there was a significant increase ($P < 0.001$) in carotene and vitamin A following interruption. No statistical differences were found for the control group. The trend for carotene after interruption was negative while that for vitamin A was positive. A calculation of the total amount of carotene and vitamin A secreted showed no significant differences between the average values before interruption with those following interruption.

The average protein, fat, and ash content (fig. 1 and 4) increased after interruption, while the average lactose content decreased. The differences between the average values before interruption and after interruption showed significance only for lactose ($P < 0.001$) and for ash ($P < 0.05$). However, a comparison within treatments of the average values before and after interruption gave highly significant differences ($P < 0.01$ in the case of protein and $P < 0.001$ in the case of fat and ash). Both protein and ash showed negative trends after interruption, and lactose a positive trend. Interruption of milking caused significant decreases ($P < 0.01$) in the total amounts of these nutrients.

Although not included in the data presented, aliquot daily samples represent-

ing the fourth, fifth, seventh, and ninth days after interruption were obtained from seven of the cows in which milking was interrupted. The amount of milk, the specific gravity, the per cent fat and the per cent protein had by the ninth day returned to the same average levels as those found before interruption of milking. Such is not the case, however, with the per cent lactose, the per cent ash and the carotene and vitamin A content expressed as micrograms per cent or per gram of fat.

The levels of blood serum calcium (table 1) were increased by interruption of milking. During the interruption of milking the average serum calcium levels were higher ($P < 0.05$) in the interrupted milking group than in the controls. There were no treatment differences prior to and after interruption of milking.

DISCUSSION

Investigation in the field of interrupted milking prior to this study has indicated that, when the removal of all or part of the milk from the mammary

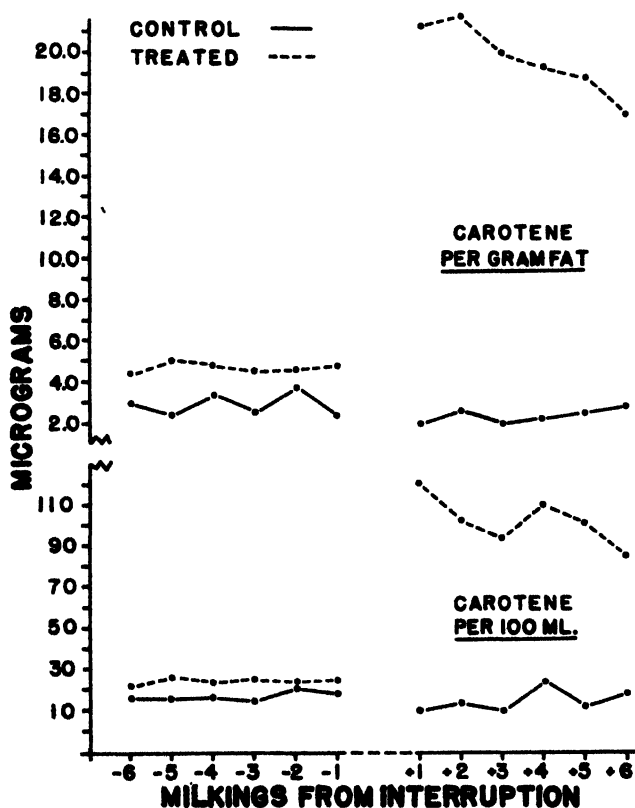


Fig. 2. The effect of interruption of milking on the carotene content of milk.

gland has been interrupted for a period of time and then resumed, changes occur in the concentration of the proximate constituents. These changes have been attributed to resorption of certain of these constituents. Previous work has utilized the individual cow's udder as an experimental unit and a control unit. The possibility of stimulatory effects resulting in higher intramammary pressures than in the non-stimulated udder probably were experienced, if the "let-down" mechanism of Ely and Peterson (7) is accepted.

The effect of interruption of milking on the concentration of the proximate constituents as reported herein is essentially in agreement with the data in the literature (1, 3, 4, 8, 12, 13, 14, 16). Examination of the total amount of these nutrients secreted after interruption would tend to support the view that resorption does take place upon interruption of milking. A more critical experiment certainly is indicated before this view can be accepted fully for all of the proximate constituents and their component parts.

In the case of carotene and vitamin A, the increase in concentration was marked, both when expressed as γ per 100 ml. of milk and γ per gram of fat. Of further interest is the positive trend in the concentration of vitamin A fol-

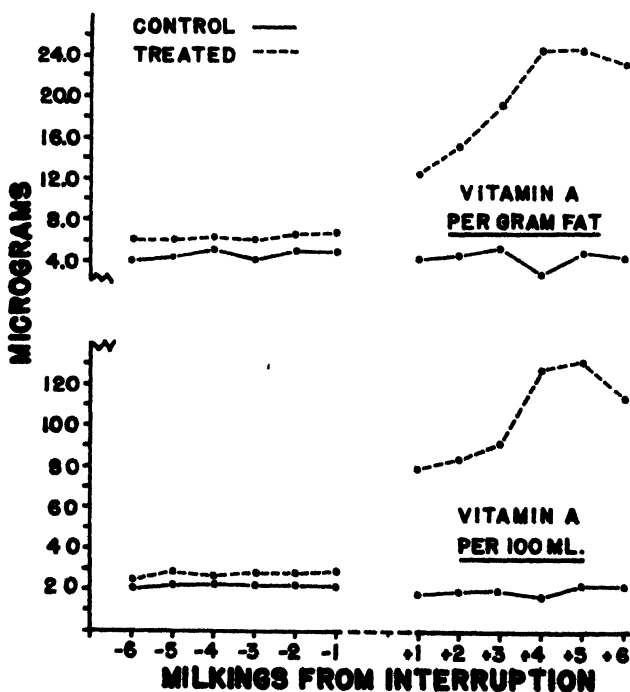


FIG. 3. The effect of interruption of milking on the vitamin A content of milk.

lowing interruption of milking which suggests that, at least under these particular conditions, the secretion of vitamin A may be independent of that of fat. The

absence of significant differences between the total amount of both carotene and vitamin A secreted before and after interruption of milking is in contrast to that found for the proximate constituents, where a marked decrease occurred.

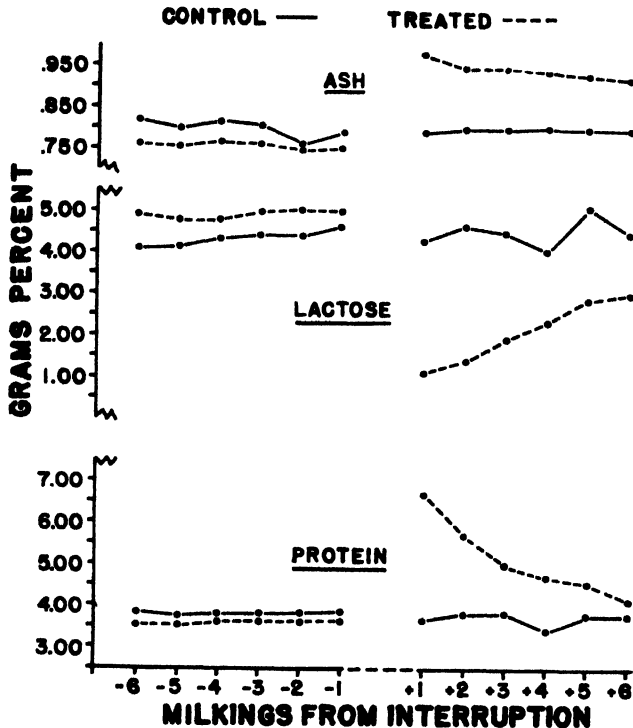


FIG. 4. The effect of interruption of milking on the per cent protein, per cent lactose, and per cent ash of milk.

Porcher (12), and Brown *et al.* (1) have pointed out that interruption of milking will result in a secretion similar to that of colostrum. The data presented in this paper would not support such a view. Colostrum from the first milking postpartum of cows fed a ration similar to that in this experiment (6) had concentrations of lactose, fat, and ash, only, which approached those values reported in the data in this experiment. In colostrum the average grams per cent protein was 12.2 and the average micrograms per cent carotene and vitamin A were 272 and 268. These values were two-fold greater than those found after interruption of milking.

The increase in blood serum calcium during the interruption of milking is similar to the report of Neidermeier and Smith (10), who found in cows not milked postpartum a similar rise 3 to 4 days after parturition. Whether this rise in serum calcium is due to resorption of calcium or to hormonal effects needs investigation.

SUMMARY

The effect of interruption of milking for a 10-day period during lactation on the carotene, vitamin A, and proximate composition of milk and on the calcium level of blood serum has been studied for 18 cows. The data indicate that interruption of milking results in significant increases in the concentration of carotene and vitamin A. With the exception of lactose, the proximate constituents also increased in concentration; lactose content decreased. The total amount of these nutrients secreted for the first 3 days after interruption was significantly lower for the proximate constituents, but no appreciable differences were noted for carotene and vitamin A. Interruption of milking, resulted in a significant elevation of blood serum calcium during the period of interruption.

ACKNOWLEDGMENTS

The authors are grateful to F. Warren and G. Farrington for the care of the experimental animals and to Misses R. J. Caverno, M. W. Dicks, and J. H. Kramer and to J. Satchell and R. J. Slate for technical assistance at various times during the course of the experiment. Further acknowledgment is due C. I. Bliss, Storrs Agricultural Experiment Station Biometrician, for suggestions in the statistical analyses of the data and to A. A. Thibault, Foreign Language Department, University of Connecticut, for aid in translation of the foreign publication.

REFERENCES

- (1) BROWN, W. R., PETERSON, W. E., AND GORTNER, R. A. Intramammary Duct Injections in the Study of Lactose Formation. *J. Dairy Sci.*, 19: 243-256. 1936.
- (2) CLARK, E. P., AND COLLIP, J. B. A Study of the Tisdall Method for the Determination of Blood Serum Calcium with Suggested Modifications. *J. Biol. Chem.*, 63: 461. 1925.
- (3) DAVIDSON, F. A. The Effect of Incomplete Removal of Milk from the Udder on the Quantity and Composition of the Milk Produced During the Intermediate Subsequent Milkings. *J. Dairy Sci.*, 7: 267-293. 1924.
- (4) DAVIDSON, F. A. Experimental Disturbances in Milk Secretion of the Cow. *J. Agr. Research*, 33: 873-885. 1926.
- (5) EATON, H. D., JOHNSON, B. E., MATTERSON, L. D., AND SPIELMAN, A. A. The Carotene and Vitamin A and Proximate Composition of Portions of the First Milking Postpartum. *J. Dairy Sci.*, 32: 587-595. 1949.
- (6) EATON, H. D., JOHNSON, B. E., SPIELMAN, A. A., MATTERSON, L. D., AND NEZVESKY, L. Prepartum Milking. II. The Effect of Prepartum Milking on the Carotene, Vitamin A and Proximate Composition of Colostrum. *J. Dairy Sci.*, 32: (in press). 1949.
- (7) ELY, F., AND PETERSON, W. E. Factors Involved in the Ejection of Milk. *J. Dairy Sci.*, 24: 221-223. 1941.
- (8) JACKSON, L. C., AND BOTHERA, C. H. I. Milk—Its Milk Sugar, Conductivity and Depression of Freezing Point. *Biochem. J.*, 8: 1-27. 1914.
- (9) MOORE, L. A., AND ELY, F. Extraction of Carotene from Plant Material. A Rapid Quantitative Method. *Ind. Eng. Chem., Anal. Ed.*, 13: 600-601. 1941.
- (10) NEDERMEIER, R. P., AND SMITH, V. R. A Study of Some Blood Constituents of Cows Not Milked Following Parturition (Abstract). *J. Dairy Sci.*, 31: 684-685. 1948.
- (11) NELSON, W. L., LOOSLI, J. K., LOFGREN, G., AND YAGER, N. Carotene Retention in Alfalfa Meal. Effect of Blanching, Packaging and Storage Temperature. *Ind. Eng. Chem.*, 40: 2196-2198. 1948.

- (12) PORCHER, CH. Lactee' retention. *Ann. fals. et fraudes*, 12: 329-343. 1919.
- (13) RAGSDALE, A. C., TURNER, C. W., AND BRODY, S. The Rate of Milk Secretion as Affected by an Accumulation of Milk in the Mammary Gland. *J. Dairy Sci.*, 7: 249-261. 1924.
- (14) RIGOR, T. V. The Effect of Pressure in the Udder Upon the Rate and Quality of Milk Secretion. Unpublished Ph.D. thesis, University of Minnesota, St. Paul. 1930.
- (15) SNEDECOR, G. W. *Statistical Methods*. 4th ed. Iowa State College Press, Ames, Iowa. 1946.
- (16) WAYNE, R., ECKLES, C. H., AND PETERSON, W. E. Drying Up Cows and the Effect of Different Methods Upon Production. *J. Dairy Sci.*, 16: 69-78. 1938.

THE VALUE OF MILK REPLACEMENTS IN THE RATIONS OF DAIRY CALVES^{1, 2}

J. B. WILLIAMS AND C. B. KNOTT

The Pennsylvania Agricultural Experimental Station

INTRODUCTION

Numerous attempts have been made to find a method of raising young dairy calves on a minimum amount of whole milk. Several investigators (1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17) have demonstrated that calves can be raised on limited quantities of milk.

The relative cost of fluid milk in some areas has encouraged the sale and use of various products in supplementing or replacing whole milk. Some of the materials used are dried skim milk, dried whey, distillers dried solubles, soluble blood flour, oat flour, fish meal, vitamin supplements and other animal and vegetable by-products. Shoptau (18) found that soybean flour was not a satisfactory substitute for milk. Wiese *et al.* (21) concluded that a synthetic milk preparation must be supplemented with riboflavin to give normal growth. Trimberger (20) has reported that distillers dried solubles can be used to replace half of the milk normally fed to calves at 3 wk. of age. Gullickson *et al.* (4) observed that calves fed vegetable oils or animal fats did not gain weight or appear as thrifty as calves fed whole milk.

The purpose of this investigation was to obtain additional information relative to the value of mixtures of some commonly used animal and vegetable products when fed to dairy calves as replacements for milk.

EXPERIMENTAL PROCEDURE

Fifty Holstein male calves obtained from state institutional herds, were divided into five comparable groups on the basis of body weight, height at the withers and chest circumference. The calves were put on the experiment not later than the fourth day following birth. Groups I, II, III and IV were fed the replacement formulae shown in table 1. The remaining ten calves constituted the control group and were fed 300 lb. of whole milk including colostrum. All calves were fed from open pails placed in the concentrate feed box located 10 inches above the floor of the pen.

Received for publication July 26, 1949.

¹ Authorized for publication as paper no. 1528 in the Journal Series of The Pennsylvania Agricultural Experiment Station. This work was supported in part by the Cooperative G. L. F. Exchange, Inc., Ithaca, N. Y. and National Distillers Products Corporation of N. Y. with the cooperation of The Distillers Feed Research Council of Cincinnati, Ohio. The sulfathalidine used in this trial was supplied by Sharp and Dohme, Inc., Glenolden, Pa.

² The data contained in this publication are from a thesis to be submitted by the senior author to the Graduate School of The Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of philosophy.

TABLE 1
Milk replacement formulae

Ingredient	I	II	III	IV
	(lb.)	(lb.)	(lb.)	(lb.)
Dried skimmilk	40	50	50	30
Dried whey	20	10	10	10
Ground beet pulp	20	10		10
Corn distiller's dried solubles		10	10	10
Blood flour			10	10
Fish meal				10
Dextrose	7.75	7.75	7.75	7.75
Oat flour	5.00	5.00	5.00	5.00
Brewer's dried yeast	4.90	4.90	4.90	4.90
Irradiated yeast (9F)	0.10	0.10	0.10	0.10
Stabilized Vitamin A feed ^b	2.20	2.20	2.20	2.20
Minerals ^c	0.042	0.042	0.042	0.042
Daily allowance	1.0	1.0	1.0	1.0
Est. daily intake of dicalcium phosphate (lb.)	0.1846	0.2195	0.301	0.2888
Est. daily intake of Ca (g.)	3.80	3.83	3.90	5.36
Est. daily intake of P (g.)	3.05	3.79	4.04	4.62
Cost ^a per calf to 8 wk. of age	\$4.69	\$5.12	\$5.76	\$5.01

^a Cost based on retail feed prices in Aug., 1948.

^b 220,000 USP units of vitamin A/lb.

^c Mineral mixture: Ferric citrate ($\text{FeC}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$) 56.57%
 Cupric sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) 19.73%
 Manganese sulfate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$) 21.59%
 Cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) 2.11%

The calves in groups I, II, III and IV were fed the mixtures at 100° F. according to the following schedule:

Birth to 4th day

Dam's milk

5th to 7th day

2.5 lb. whole milk, 0.25 lb. milk replacement, 2 lb. water³

8th to 10th day

1 lb. whole milk, 0.5 lb. milk replacement, 4 lb. water³

11th to 35th day

0.5 lb. milk replacement, 5 lb. water³

36th to 49th day

0.5 lb. milk replacement, 6 lb. water³

50th to 56th day

0.5 lb. milk replacement, 6 lb. water (once daily).

The calves in group V were fed whole milk which averaged about 3.8 per cent fat according to the following schedule: birth through 10th day—8 lb. per day, 11th through 24th day—10 lb. per day, 25th through 28th day—8 lb. per day and 29th through 36th day—6 lb. per day.

The calves of all groups were fed a good quality alfalfa hay *ad libitum* from birth to the conclusion of the 16-wk. trial. Each calf was provided with calf starter *ad libitum* until each was consuming the maximum of 6 lb. daily. The

³ Twice daily.

calf starter was prepared as follows: yellow corn meal 406.5 lb., wheat bran 300 lb., crushed oats 400 lb., linseed oil meal 140 lb., soybean oil meal 280 lb., dehydrated alfalfa meal 140 lb., cane molasses 100 lb., dried skimmilk 100 lb., dried corn distiller's solubles 100 lb., irradiated yeast (9F) 0.5 lb., dicalcium phosphate 10 lb., ground limestone 10 lb., iodized salt 10 lb., and Vitamin A feeding oil 3 lb. (2,724,000 USP units of A per pound).

The barn was artificially lighted and ventilated, and thermostatically maintained at a temperature of 65° F. by means of steam heat. Each calf was placed in an individual pen which was equipped with a salt block, water bowl, concentrate feed box and hay manger. The calves were placed at random throughout the barn so as to prevent positional effects. Growth measurements were taken by the same person each week at the same time and in the same order with respect to the body weight, height at the withers and chest circumference. Daily observations of the conditions of the feces of each calf were made by the same person throughout the trial. Photographs of each calf were taken at birth, 4, 8, 12 and 16 wk. of age. Upon arrival each calf was given orally, 5 g. of sulfathalidine and an additional 5 g. at each of the next three successive feedings.

EXPERIMENTAL RESULTS

TABLE 2

Summary of growth^a and cost data

Group	Body weight		Withers height		Chest cir.		Cost ^b
	8 wk.	16 wk.	8 wk.	16 wk.	8 wk.	16 wk.	16 wk.
I	0.33	1.07	0.07	0.10	0.02	0.07	0.20
II	0.78	1.41	0.13	0.15	0.06	0.09	0.18
III	0.84	1.35	0.10	0.14	0.07	0.09	0.19
IV	0.89	1.32	0.12	0.14	0.08	0.09	0.19
V	0.90	1.36	0.12	0.14	0.06	0.09	0.22

^a Expressed as mean daily gains.

^b Based on retail feed prices in Aug., 1948 per lb. gain.

The summary of the growth data for the 16-wk. trial is presented in table 2. It would appear from this summary that the calves of group II were slightly superior to the other groups, but when the data were analyzed statistically according to the methods of Snedecor (19) there were no significant differences between groups II, III, IV and V in terms of body weight, height at the withers and chest circumference. However, group I was significantly poorer than all other groups on the same basis. The cost per lb. of gain in body weight was somewhat lower for groups II, III and IV than for V. The high cost of fluid milk, even though fed in limited amounts, was the reason for the higher cost in group V. Gain was less costly in group I as compared to group V, but rate of gain was considerably less also.

The only loss was one calf in group I which died at 30 days of age. The individuals in this group suffered frequent and prolonged scouring until about 30 days of age, with two calves being unable to stand on their feet for several days



FIG. 1. Calf 418, a typical individual in group I at 4 wk. of age.



FIG. 2. The extent of the loss of hair from calf 418 can still be seen at 16 wk. of age, but complete recovery is apparent.

at that age. All calves in group I incurred considerable hair loss over the entire body, muscular incoordination and weakness, profuse lacrimation and papilladema but maintained their appetites even when unable to stand. Fig. 1 is a photograph of calf no. 418, a representative calf of group I, at 4 wk. of age, while fig. 2 is the same calf at 16 wk. of age. The hair loss began on the 18th to 21st day of age and continued until the calves began to ruminate at an average of 35 days of age, at which time the hair loss stopped and new hair began to grow in. Three calves in group II suffered some loss of hair about the forehead and one calf in group IV suffered considerable loss of hair over the entire body. There was no hair loss in groups III and V.

The amounts of hay and grain presented are those actually consumed, as all refusals were weighed back. The average consumption of the calf starter was similar in all groups except group I. This group consumed an average of 301 lb. of starter and the other four groups varied from 351 lb. to 363 lb. Average hay consumption per calf for groups II, IV and V were 150, 144 and 151 lb., respectively; calves in group I each consumed an average of 112 lb. and calves in group III each 172 lb.

Scouring was not a problem in any of the groups with the exception of group I. When a scouring condition persisted for more than 24 hr., a 10 g. dose of sulfathalidine was given and another 5 g. at each of the next two successive feedings. The drug was administered orally in 5 g. capsules and effectively controlled all cases except those occurring in group I. As high as 40 g. were given to calves in group I without any apparent relief from the condition. The teeth of four of the calves in group I were loose and greatly discolored with red, tender gums. Some of the calves suffered tongues swollen so badly that swallowing was difficult, even though the calf was hungry. The mouth was very tender, making it painful to work the trip in the water bowls; the water bowls had to be operated manually by the feeder until the condition cleared up.

The feces of all of the calves on the replacement formulae were very dark and rather soft until a considerable amount of hay and starter were being ingested, at which time they were similar in all respects to the feces of the milk-fed calves.

The general appearance of the animals in groups I, II and IV was not as satisfactory as the calves in groups III and V; however, at 60 days of age there was very little difference in the groups. At the end of the 16-wk. trial several of the calves from group I were comparable to the other groups in all respects.

Palatability was not a problem with any of the formulae, as calves 4 days old easily were taught to take the mixtures from the pail. Mix I had a greater tendency to settle out than the others, especially when the very young calves were slow in consuming the mixture. The other mixes went into suspension very readily and remained so until the calf had consumed the entire amount. One animal in group III persistently refused to take the replacement unless aided by the feeder.

In an effort to correct the symptoms occurring in group I additional calves similarly were fed and managed but received in addition daily by oral administration the following; calf 411A—50 mg. ascorbic acid, calf 411B—10 mg. biotin,

calf 411C—0.2 lb. vitamin free casein, calf 411P—20 mg. calcium pantothenate, calf 411R—5 mg. riboflavin, calf 481—10,000 USP units of vitamin A, calf 480—2.7 mg. of 70 per cent choline chloride, calf 482—0.7 gm. l-cystine, calf 483—3.5 gm. methionine, and calf 485—received the latter three in combination in identical amounts daily. These supplements failed to effect the general pattern of hair loss and other symptoms observed in group I.

Sixteen additional calves have been fed a milk replacement similar to that fed to group III except that it contained 0.22 lb. of stabilized vitamin A feed (2,274,000 USP units per lb.) which replaced 2.20 lb. of stabilized vitamin A feed (220,000 USP units of vitamin A) and 2.50 lb. of dicalcium phosphate in addition. Similar response in growth and general appearance was obtained. This modified formula is now being used in a field trial with a relatively large number of calves and is giving satisfactory results.

SUMMARY

1. A milk replacement formula is presented which produced calves equal in rates of growth and general appearance to milk fed controls under the conditions of these experiments.

2. A replacement containing 20 per cent beet pulp was found to produce certain deleterious effects, under the conditions of this trial. These symptoms were not prevented by the oral administration of ascorbic acid, biotin, vitamin free casein, calcium pantothenate, riboflavin; choline-chloride, l-cystine, methionine or vitamin A.

The cooperation of V. A. Houston and C. R. Barber of the Pennsylvania Department of Welfare in providing the calves used in this project is sincerely appreciated. This was a cooperative project with the Department of Animal Husbandry of Cornell University.

REFERENCES

- (1) BERRY, M. H. Use of Skimmilk Powder, Blood Flour, and Fish Meal in Grain Rations for Calves. Md. Agr. Expt. Sta. Bull. 354. 1933.
- (2) DAVIS, R. N., AND CUNNINGHAM, W. S. Raising Calves on the Minimum Amount of Milk. Ariz. Agr. Expt. Sta. Bull. 111. 1923.
- (3) FLIPSE, R. J., HUFFMAN, C. F., DUNCAN, C. W., AND THORP, F., JR. A Comparison of Corn Starch, Dextrin and Corn Sugar as the Principal Carbohydrate Source in Synthetic Rations for Calves (Abs.). J. Dairy Sci., 31: 674. 1948.
- (4) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Calves. J. Dairy Sci., 25: 117-128. 1942.
- (5) HATHAWAY, J. L., TRIMBERGER, G. W., AND DAVIS, H. P. The Use of Dried Whey and Blood Meal in the Raising of Calves on Limited Amounts of Milk. Neb. Agr. Expt. Sta. Research Bull. 132. 1943.
- (6) HAYWARD, H. The Rearing of Calves on Milk Substitutes. Pa. Agr. Expt. Sta. Bull. 60. 1902.
- (7) INGHAM, L. W., MEADE, D., AND BERRY, M. H. Calf Feeding Investigations. I. Raising Dairy Calves on Nurse Cow, Whole Milk, Remade Skimmilk and Calf Meal. Md. Agr. Expt. Sta. Bull. 319. 1930.
- (8) JACOBSON, N. L., AND CANNON, C. Y. Soybean Oil-filled Milks for Feeding Young Dairy Calves. J. Dairy Sci., 30: 587. 1947.

- (9) JOHNSON, P. E., LOOSLI, J. K., AND MAYNARD, L. A. Purified Diet Studies with Calves. *J. Dairy Sci.*, 23: 553-554. 1940.
- (10) KRAUSS, W. E., AND CRAWFORD, C. H. Powdered Skimmilk as a Feed for Dairy Calves. *Ohio Agr. Expt. Sta. Bi-monthly Bull.* 14: 49-56. 1929.
- (11) KRAUSS, W. E., AND MONROE, C. F. Dry Feed Systems of Raising Calves. *Ohio Agr. Expt. Sta. Bull.* 548. 1935.
- (12) MAYNARD, L. A., AND NORRIS, L. C. A System of Rearing Dairy Calves with Limited Use of Milk. *J. Dairy Sci.*, 6: 483-508. 1923
- (13) NORTON, C. L., AND EATON, H. D. Dry Calf Starters for Dairy Calves. N. Y. (Cornell) *Agr. Expt. Sta. Bull.* 835. 1946.
- (14) RUPEL, I. W. Raising the Dairy Calf. *Wis. Agr. Expt. Sta. Bull.* 404. 1929.
- (15) SAVAGE, E. S., AND CRAWFORD, C. H. Dry Concentrates as a Partial Substitute for Whole Milk in Calf Ration. N. Y. (Cornell) *Agr. Expt. Sta. Bull.* 622. 1935.
- (16) SAVAGE, E. S., AND MCCAY, C. M. The Nutrition of Calves: A Review. *J. Dairy Sci.*, 25: 595-650. 1942.
- (17) SCHABINGER, J. R., AND KNOTT, C. B. The Value of Distiller's Dried Solubles and Dried Grains with Solubles in the Ration of Dairy Calves. *J. Dairy Sci.*, 30: 585-586. 1947.
- (18) SHOPTAU, L. Soybean Flour as a Substitute for Cow's Milk in Feeding Dairy Calves. *J. Dairy Sci.*, 19: 95-99. 1936.
- (19) SNEDECOR, G. W. *Statistical methods*, 4th ed. The Iowa State College Press, Ames. 1946.
- (20) TRIMBERGER, G. W. Agrisol Distiller's Dried Solubles for Poultry, Hogs, Sheep and Cattle. Mimeo. Farm Crops Processing Corp., Omaha, Neb.
- (21) WIESE, A. C., JOHNSON, B. C., MITCHEL, H. H., AND NEVENS, W. B. Synthetic Rations for the Dairy Calf. *J. Dairy Sci.*, 30: 87-94. 1947.

THE USE OF *CANDIDA LIPOLYTICA* CULTURES IN THE MANUFACTURE OF BLUE CHEESE FROM PASTEURIZED HOMOGENIZED MILK¹

C. E. PARMELEE AND F. E. NELSON

Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

The pasteurization of milk for the manufacture of blue cheese has received increasing interest since the enactment of various state laws requiring pasteurization or minimum curing periods for cheese. Pasteurization destroys the normal milk lipase which is responsible for much of the fat breakdown required in the development of the flavor of blue cheese. One logical solution to the problem is the substitution of another lipase for the milk lipase. The present study is an attempt to substitute, for the lipase of milk, the lipases produced in the cheese by certain microorganisms added as pure cultures to the milk from which cheese is made.

HISTORICAL

The manufacture of blue cheese from pasteurized milk was mentioned by Goss, Nielsen and Mortensen (8) in 1935. Cheese made from pasteurized milk did not develop as much surface growth of molds and bacteria, did not have as much flavor and did not become as sweet during curing as did the cheese made from raw milk. Lane and Hammer (11) found that the flavor and color of cheese made from pasteurized homogenized milk were inferior to those of cheese made from raw homogenized milk.

Irvine (10) reported that the addition of 0.5 to 1.0 g. of lipase preparation to 100 lb. of raw milk accelerated fat hydrolysis but always gave a bitter flavor in blue cheese. The lipase used was identified as steapsin (13). Coulter and Combs (4) found that this enzyme would give the same amount of flavor in 5 months' curing that had been obtained previously in 12 months' curing of raw milk cheese; however, the bitter flavor was present. They made several lots of blue cheese from pasteurized milk to which 2 g. of steapsin per 100 lb. of milk had been added. These lots of cheese were not inoculated with mold and were not pierced but they had the rank flavor of butyric acid to the exclusion of all other flavors up to 15 months. The volatile acid values were very high on these lots of cheese.

By adding controlled amounts of desiccated sheep mammary tissue to blue cheese curd made from pasteurized milk, Lane and Hammer (12) were able to make a cheese which would ripen in the same length of time required for cheese made from raw milk containing homogenized fat. Babel and Hammer (1) found that addition of steapsin to the curd or to the milk resulted in cheese having a bitter flavor and somewhat gray color. Desiccated mammary tissue produced cheese having more flavor than the control cheese, and the cheese, if made from pasteurized homogenized milk, were quite satisfactory after ripening for 3 months.

Received for publication July 22, 1949.

¹ Journal paper no. J1672 of the Iowa Agricultural Experiment Station, Project 895.

Cheese made from pasteurized milk, to which various enzymes had been added, were improved by homogenization of the milk.

The use of the cell-free extract of *Mycotorula lipolytica* (now termed *Candida lipolytica*) in pasteurized homogenized milk for blue cheese already has been reported by Peters and Nelson (14).

The lipolytic activity of a number of microorganisms has been investigated. The lipolytic activity of *Pseudomonas fragi* was studied by Hussong *et al.* (9) by Fouts (5). *Achromobacter lipolyticum* was found by Collins (3) to produce rancidity in butterfat, corn oil and tributyrin and tallowiness in olive oil and triolein. Fouts (5, 6, 7), in studying the effect of the growth of organisms on the acidity of the fat in cream and butter, found that *Penicillium roqueforti*, *Oospora lactis*, *M. lipolytica*, *A. lipolyticum*, *Alcaligenes lipolyticus* and *Pseudomonas fluorescens*, but not *P. fragi*, caused increases in the acid numbers of the fat when inoculated into sterilized cream. *M. lipolytica* was the only organism in the group which showed increased growth in the presence of butter culture organisms. Of this group of organisms, *M. lipolytica* and *A. lipolyticus* were found to give the greatest increases in the total volatile acidity of butterfat.

EXPERIMENTAL

The milk used was normal mixed herd milk, pasteurized at 143–147° F. for 30 min., cooled to about 130° F., homogenized at 1,700 lb. pressure and cooled to 40–45° F. The milk was made into cheese immediately or was held below 40° F. until the following day. The milk in the vat was heated to 89–90° F. and 1 per cent of lactic culture was added. After 30 min. ripening, the milk was set with rennet used at the rate of 90 ml. per 1,000 lb. of milk in lots 4 to 51 and 130 ml. per 1,000 lb. of milk in all other lots. The curd was cut with 0.5 inch curd knives 70 min. after rennet addition. The cut curd was allowed to stand for about 30 min. and then was stirred gently every 20 to 30 min. until firm enough to hoop. Heat was applied to the jacket of the vat at the stirring time, whenever necessary to keep the temperature at 90° F. inside the vat. The curd was firm enough to hoop at 2.0 to 2.5 hr. from the time it was cut, and the whey acidity usually had increased 0.04 to 0.06 per cent. One per cent salt and 0.01 per cent mold powder were added to the curd at the time of hooping. Dry salt was rubbed on the surface of the cheese every day for 4 or 5 days, until 5 per cent salt had been used. The day after salting was completed, the cheese of lots 4 to 119 were dipped in flexible cheese coating and pierced about 50 times with a wire needle, 0.1 inch in diameter. All other lots were pierced but not coated. The cheese was cured at 48 to 52° F. at a relative humidity of 90 to 95 per cent.

Each trial consisted of a control lot of cheese and three lots in which one factor was varied, all made from separate 115-lb. portions of the same milk. The lots of cheese were scored at various intervals for mold growth, flavor development and defects in flavor. Each item had a range of score from 0 to 10 in which 10 was the most perfect score. This should be remembered when considering the defect score, because the higher the defect score, the less serious was the defect. The total volatile acidity of the cheese was determined by the method of Lane

and Hammer (11). Fat acidity was determined by the method of Breazeale and Bird (2) after the fat was obtained and purified by the method of Lane and Hammer (11).

Preliminary trials were made to determine the species of microorganism, the type of culture and the amount of culture that should be used to improve the flavor of blue cheese made from pasteurized milk. Cultures of each of the four lipolytic microorganisms, *Candida lipolytica* (strain 846), *Alcaligenes lipolyticus*, *Achromobacter lipolyticum* and *Pseudomonas fragi* were prepared by inoculating three portions of 18 per cent sterilized homogenized cream with the organisms and incubating one at 30° C. for 24 hr. and two at 30° C. for 48 hr. One of the 48-hr. cultures was sterilized in the autoclave at 15 lb. pressure for 30 min. before it was used in milk for cheese. The cultures were added to milk at the rate of 555 g. (1.2 per cent) per 100 lb. of milk. That amount of cream contained 100 g. of butterfat. The total volatile acidities, fat acidities, scores and comments for the cheese made from milk to which these cultures were added are presented in table 1. Of the four organisms used, *C. lipolytica* gave the largest and most consistent increase in fat acidity and total volatile acidity. The flavor scores for the lots of cheese made with this culture were not high, possibly due to the deficiency of mold growth, but the increase in flavor score over that of the control cheese was greatest for the cheese made with this culture. It was the only culture criticized for producing excess fatty acids in the cheese, which indicated that 1.2 per cent of an active 48-hr. culture of this organism was too much to use. The sterilized 48-hr. culture did not improve the cheese.

Based upon the results of this experiment, another trial was made in which different amounts of a culture of each of the four organisms grown for 48 hr. at 30° C. in sterile homogenized milk were added to each of three lots of milk for cheese. The amounts of culture added and the results obtained are presented in table 2. The improvement of the cheese by the use of a culture of *C. lipolytica* is shown more conclusively by these results. It is the only organism of the four used that increased the total volatile acidity and flavor score appreciably. The use of this culture, at all percentages tried, gave the highest total volatile acidities and flavor scores at 12 wk. of any of the cultures used. The cultures of *C. lipolytica* prepared in homogenized milk appeared to have improved the cheese as much as those prepared in homogenized 18 per cent cream. Fat acidity determinations were discontinued because of the difficulty encountered in extracting the fat and because the values did not appear to be as closely related to flavor scores as were the total volatile acidity values.

As *C. lipolytica* was the only organism of the four used in preliminary studies that consistently improved the flavor and increased the volatile acidity values of blue cheese made from pasteurized homogenized milk, this organism was studied further to determine the effect of various strains of the organism upon the ripening of the cheese. Cultures of eleven strains of the organism from various sources were grown in sterilized homogenized milk for 48 hr. at 30° C., and were added to milk for blue cheese at the rate of 0.3 per cent. Second and third trials were made with some of the strains which gave good results in the first trials.

TABLE 1
The effect of the addition of various cultures of four lipolytic organisms to pasteurized homogenized milk for blue cheese manufacture

Lot no.	Culture in 18% cream incubated at 30° C. Culture used	Age	Total volatile acidity ^a		Fat acidity ^b		Score				Defect comment for cheese 12 wk. old		
			acidity ^a				Mold		Flavor				
			4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.			
		(hr.)											
32	None		7.80	7.60	9.05	2.68	2.5	1.5	1.5	2.0	4.5	1.5	Sour, yeasty, musty
33	<i>C. lipolytica</i>	48 ^c	5.00	12.00	4.03	7.58	5.0	2.0	5.0	2.0	7.0	1.5	Musty, yeasty (invasion)
34	<i>C. lipolytica</i>	24	7.00	13.50	7.45	8.68	2.0	2.5	4.0	4.0	4.5	4.0	Musty, sl. sour, yeasty
35	<i>C. lipolytica</i>	48	11.40	26.00	18.00	23.40	4.5	3.5	3.5	3.0	3.0	5.0	Excessive fatty acids
44	None		5.80	15.00	4.58	8.85	5.5	5.0	4.0	5.5	7.0	4.0	Musty, yeasty
45	<i>A. lipolyticus</i>	48 ^c	6.00	15.30	2.59	18.63	5.0	7.0	5.0	6.0	6.0	3.5	Unnatural, musty, yeasty
46	<i>A. lipolyticus</i>	24	6.30	16.80	2.40	26.30	7.0	5.5	5.5	6.5	7.0	3.0	Unnatural, musty, yeasty
47	<i>A. lipolyticus</i>	48	5.60	16.30	2.52	10.12	7.5	7.5	6.0	7.0	7.0	2.5	Unnatural, sour, musty, yeasty
36	None		7.00	13.36	5.74	9.23	3.0	3.5	3.0	5.0	4.0	7.0	Yeasty, sl. unclean, green
37	<i>A. lipolyticum</i>	48 ^c	8.00	15.60	6.12	26.38	2.5	5.0	3.0	7.0	3.0	8.0	Sl. musty, sl. unclean
38	<i>A. lipolyticum</i>	24	7.70	15.00	5.26	10.33	5.0	6.0	5.0	7.5	6.5	8.0	Sl. musty, sl. unclean
39	<i>A. lipolyticum</i>	48	7.00	20.60	5.88	16.58	8.0	4.5	4.5	6.0	8.0	5.0	Very musty, sl. unclean
40	None		6.90	15.00	1.40	8.18	5.0	6.0	2.5	5.0	6.0	6.0	Sour, musty
41	<i>P. fragi</i>	48 ^c	6.70	15.30	4.82	17.48	5.5	7.0	3.0	4.0	5.0	4.5	Musty, cheddar
42	<i>P. fragi</i>	24	7.00	16.80	5.14	11.98	3.5	5.0	4.5	4.0	8.5	2.0	Musty, unclean
43	<i>P. fragi</i>	48	6.70	16.30	4.50	14.22	3.0	6.0	6.0	6.0	9.0	5.0	Musty, unclean

^a Total volatile acidity expressed as ml. 0.1 N acid/200 g. cheese.

^b Fat acidity expressed as ml. 0.1 N acid/10 g. fat.

^c Culture was sterilized after incubation before addition to milk for cheese.

TABLE 2
The effect of the addition of varying amounts of homogenized milk culture of lipolytic organisms to pasteurized homogenized milk for blue cheese manufacture

Lot no.	Culture used	Cul- ture added	Total volatile acidity ^a				Score				Defect comments for cheese 12 wk. old
			Mold		Flavor		Defect				
			4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.			
(%)											
56	None		6.20	19.50	8.0	4.5	3.5	4.0	3.0	3.5	Sour, unclean
57	<i>C. lipolytica</i>	0.1	12.70	30.20	6.0	7.0	6.0	6.0	8.0	4.5	Unclean
58	<i>C. lipolytica</i>	0.3	8.60	35.50	8.0	5.0	7.0	7.0	8.0	6.5	Sl. unclean
59	<i>C. lipolytica</i>	1.0	17.60	40.90	6.5	6.5	6.5	8.0	7.5	7.5	Sl. unclean (invasion)
60	None		8.00	24.00	3.0	6.5	2.0	4.5	2.0	6.0	Sour, salty, lacking
61	<i>A. lipolyticus</i>	0.1	7.40	19.00	5.0	7.0	3.0	5.0	3.0	6.0	Lacking
62	<i>A. lipolyticus</i>	0.3	7.00	18.00	3.0	4.0	3.5	4.0	3.5	6.0	Sour, lacking
63	<i>A. lipolyticus</i>	1.0	6.80	18.20	3.0	4.5	4.0	4.0	4.0	6.0	Sour, lacking
64	None		5.50	7.80	7.0	6.5	4.0	4.5	3.0	3.5	Unnatural
65	<i>A. lipolyticum</i>	0.1	6.00	8.00	8.0	7.5	5.5	5.0	7.0	5.0	Unnatural
66	<i>A. lipolyticum</i>	0.3	5.40	8.00	4.5	7.5	3.0	5.5	5.0	6.0	Unnatural
67	<i>A. lipolyticum</i>	1.0	5.50	6.00	6.0	6.5	3.0	6.0	4.5	4.0	Unnatural, yeasty
68	None		6.60	8.50	3.0	7.0	3.0	3.5	2.5	3.5	Unnatural, sour, yeasty
69	<i>P. fragi</i>	0.1	7.10	7.70	5.5	5.0	3.5	5.0	4.0	5.0	Unnatural, sour, yeasty
70	<i>P. fragi</i>	0.3	5.90	6.60	3.5	4.5	4.5	5.0	4.0	4.5	Unnatural, sour
71	<i>P. fragi</i>	1.0	6.00	8.50	4.5	6.5	4.5	5.0	5.0	4.0	Unnatural, sour

^a Total volatile acidity expressed as ml. 0.1 N acid/200 g. cheese.

TABLE 3

The effect of the addition of 0.3 per cent culture of various strains of Candida lipolytica to pasteurized homogenized milk for blue cheese manufacture

Lot no.	Strain no.	Total volatile acidity ^a		Mold		Score		Defect		Defect comment for cheese 12 wk. old
		acidity ^a				Flavor		Defect		
		4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
76		7.40	32.00	6.5	7.0	4.0	3.5	7.0	4.0	Nutty, musty
77	47	6.50	35.00	6.5	8.5	5.5	5.5	7.0	6.5	Nutty
78	57	5.60	24.50	7.0	8.0	5.0	5.0	7.0	7.5	Sl. nutty
79	100	9.00	25.70	6.0	6.0	6.0	4.5	6.0	5.5	Sour, nutty
80		7.30	27.00	7.0	9.0	4.0	3.5	5.0	3.0	Unclean, covey, sour
81	438	8.60	27.20	7.0	7.0	5.0	5.5	4.0	5.0	Unclean
82	839	19.30	51.40	5.5	5.5	7.5	8.0	8.0	7.5	Sl. excessive volatile acids
83	840	8.30	33.20	7.0	9.0	5.0	6.0	5.0	6.0	Sl. unclean
84		6.80	35.00	5.5	7.5	4.0	4.0	7.5	4.5	Nutty, burned, unnatural
85	843	20.50	50.40	5.0	7.5	7.0	8.0	7.5	7.5	Musty
86	845	8.50	36.80	5.0	7.5	3.5	5.5	4.5	5.0	
87	846	9.60	47.00	6.0	7.5	4.5	7.0	3.5	7.5	
88		8.00	21.30	7.0	7.5	4.5	4.5	4.0	5.0	Nutty, lacks pepper
89	M.L.	13.90	43.70	5.0	9.0	5.5	8.0	8.0	8.0	Sl. excessive sharpness
90	848	13.90	39.80	6.0	6.5	7.0	7.0	7.0	6.0	Excessive sharpness, soapy
91		14.50	45.00	6.0	8.0	6.0	7.5	6.0	6.5	Excessive sharpness, al. soapy
112		7.25	13.30	5.5	6.5	4.5	5.0	5.5	4.0	Unclean, al. bitter
113	839	8.00	18.00	3.0	4.5	5.0	7.0	7.0	7.0	
114	843	7.90	14.40	4.0	3.0	4.0	4.0	6.0	4.0	Yeasty, unnatural, al. sour
115	848	7.70	16.00	5.0	5.5	5.5	5.5	7.0	5.5	Sl. sour, al. unclean
116		7.00	13.00	4.0	3.0	4.0	5.0	5.0	5.0	Sour
117	846	16.70	33.00	3.5	3.0	6.0	7.5	7.5	6.5	Sl. sour, sharp
118	100	9.20	14.70	5.0	4.5	4.0	4.5	5.0	3.0	Unclean, sour, unnatural
119	M.L.	13.40	23.40	4.0	4.0	6.0	8.0	7.5	8.0	
124		10.00	20.80	4.0	6.0	3.0	4.0	4.0	3.0	Sour, cheddary, unnatural
125	M.L.	13.50	40.70	5.0	5.0	5.5	6.5	7.5	4.5	Cheddary, soapy
126	843	10.30	19.60	3.5	5.0	4.5	5.0	7.5	4.5	Cheddary, al. bitter
127	848	10.80	26.60	6.0	5.0	4.5	7.0	5.5	6.5	Cheddary, unnatural

^a Total volatile acidity expressed as ml. 0.1 N acid/200 g. cheese

The results for all trials are shown in table 3. The various trials with a single strain were not grouped in the table because in each case the three lots of cheese should be compared with the control lot of cheese for that group.

In general, the strains of *C. lipolytica* used gave cheese which had a higher total volatile acidity and flavor score at 12 wk. than did the control cheese. Strains M. L., 848, 846 and 839 improved the cheese the most, whereas strains 47, 57, 100 and 438 had little or no effect in improving the cheese. The erratic results of the three trials with strain 843 cannot be explained satisfactorily at this time. In general, the volatile acidity values correlate with the flavor score values quite closely, indicating that the lipolytic action of the culture was at least one of the most important factors in flavor production.

DISCUSSION

The strain variation found in these trials indicates that the use of *C. lipolytica* cultures in milk for blue cheese requires careful selection of strains and adjustment of the amount of culture to use for each strain and for the intensity of flavor desired in the cheese.

The data presented tend to show some correlation of total volatile acidity to flavor score but very little correlation of fat acidity to total volatile acidity and flavor score.

The addition of a culture of a proven strain of *C. lipolytica* to pasteurized homogenized milk for blue cheese on a commercial scale would be much easier and less expensive than the preparation and use of an enzyme from the organism, or the use of a commercially prepared enzyme from the organism.

SUMMARY AND CONCLUSIONS

1. The addition of cultures of the lipolytic organisms *Alcaligenes lipolyticus*, *Achromobacter lipolyticum* and *Pseudomonas fragi* to pasteurized homogenized milk for blue cheese manufacture did not improve appreciably the flavor of the resultant cheese and did not cause any appreciable increase in total volatile acidity.

2. The addition of a culture of *Candida lipolytica* to milk for cheese making improved the flavor score and increased the total volatile acidity of the cheese.

3. Eleven strains of *C. lipolytica* from various sources differed markedly in their ability to increase the total volatile acidity and to improve the flavor of blue cheese made from pasteurized homogenized milk.

4. The results presented indicate a relationship of the total volatile acidity to the flavor score of blue cheese made from pasteurized homogenized milk.

REFERENCES

- (1) BABEL, F. J., AND HAMMER, B. W. Iowa Agricultural Experiment Station, Report on Agricultural Research for the Year Ending June 30, 1942. p. 166. 1942.
- (2) BREASNALE, D. F., AND BIRD, E. W. A study of Methods for the Determination of Acidity in Butterfat. J. Dairy Sci., 21: 335-344. 1938.

- (3) COLLINS, M. A. The Action of Lipolytic Bacteria on Some Simple Triglycerides and Some Natural Fats. Unpublished Ph.D. thesis. Iowa State College Library. Ames, Iowa. 1933.
- (4) COULTER, S. T., AND COMBS, W. B. The Use of Steapsin in the Manufacture of Blue Cheese. J. Dairy Sci., 22: 521-525. 1939.
- (5) FOUTS, E. L. Some Factors Responsible for Variations in the Acid Numbers of the Fat in Cream and in Commercial Butter. J. Dairy Sci., 23: 245-258. 1940.
- (6) FOUTS, E. L. Effect of Lactic Acid on the Hydrolysis of Fat in Cream by Pure Cultures of Lipolytic Microorganisms. J. Dairy Sci., 23: 303-306. 1940.
- (7) FOUTS, E. L. Relation of Volatile Acidity of Butterfat to Rancidity. J. Dairy Sci., 23: 307-314. 1940.
- (8) GOSS, E. F., NIELSON, V., AND MORTENSEN, M. Iowa Blue Cheese. Iowa Agr. Expt. Sta. Bull. 324. 1935.
- (9) HUSSONG, R. V., LONG, H. F., AND HAMMER, B. W. Classification of the Organisms Important in Dairy Products. II. *Pseudomonas fragi*. Iowa Agr. Expt. Sta. Research Bull. 225. 1937.
- (10) IRVINE, O. R. Canadian "Blue" Cheese. Can. Dairy Ice Cream J., 7: 19-22. 1938.
- (11) LANE, C. B., AND HAMMER, B. W. Some Factors Affecting the Ripening of Blue (Roquefort-type) Cheese. Iowa Agr. Expt. Sta. Research Bull. 237. 1938.
- (12) LANE, C. B., AND HAMMER, B. W. Iowa Agricultural Experiment Station. Report on Agricultural Research for the Year Ending June 30, 1941. Part I. Pp. 147-148. 1941.
- (13) ONTARIO, CANADA, DEPARTMENT OF AGRICULTURE. Sixty-second Annual Report of the Ontario Agricultural College and Experimental Farm. 1936: 57. 1937.
- (14) PETERS, I. I., AND NELSON, F. E. The Influence of *Mycotorula lipolytica* upon the Ripening of Blue Cheese Made from Pasteurized Homogenized Milk. J. Dairy Sci., 31: 611-618. 1948.

THE USE OF A MOLD-ENZYME PREPARATION IN THE MANUFACTURE OF BLUE CHEESE FROM PASTEURIZED HOMOGENIZED MILK¹

C. E. PARMELEE AND F. E. NELSON

Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

The production of enzymes by molds and the changes produced by these enzymes in dairy products have been recognized for some time. As early as 1910, Dox (2) reported that Laxa had noted that butter inoculated with *Penicillium glaucum* developed considerable acidity. Currie (1) concluded that during the ripening of Roquefort cheese, considerable fat was hydrolyzed by the water-soluble lipase produced by *Penicillium roqueforti*, and the characteristic peppery flavor and burning effect of the cheese on the tongue and palate were considered due to the caproic, caprylic and capric acids and their readily hydrolyzable salts which had accumulated. He noted that *P. roqueforti* will grow in Czapek's medium when sucrose is replaced by pure butterfat, tributyrin, ethyl butyrate, glycerin, butyric acid, or ammonium butyrate as the source of carbon. Thus, the mold not only has the power to hydrolyze fats but is able to utilize their components, also.

In cheese, it is believed that the enzyme diffuses beyond the growth zone of the mold and hence free fatty acids accumulate. Kirsh (4) reported that the water-soluble lipase of *Penicillium oxalicum* is highly non-specific, as it brings about almost the same amount of hydrolysis of esters, triglycerides of low molecular weight and a variety of emulsified oils. Fouts (3) observed that growth of *P. roqueforti* in sterilized cream appreciably increased the acidity of the fat.

Naylor, Smith and Collins (5) obtained maximum esterase production by *P. roqueforti* on Czapek's medium in which the sodium nitrate was replaced by 0.1 N ammonium chloride and to which 0.50 ml. of ethyl butyrate was added per 1,000 ml., the reaction being adjusted to pH 4.5.

Thibodeau and Macy (7) found that *P. roqueforti* does not grow readily in a medium with an oxidation-reduction potential of over 400 millivolts. The addition of 0.1 per cent agar to Czapek's solution reduced the oxidation-reduction potential below 400 millivolts; addition of peptone or milk further reduced it. Presence of sugar in a medium tended to reduce lipase production by *P. roqueforti*. Maximum lipase was produced in Czapek's medium without sugar, but with 3.0 g. peptone and 3.0 g. butterfat per liter. The optimum activity of this lipase was over the pH range from 5.3 to 7.5, when the substrate was a 3.0 per cent butter oil emulsion in the presence of an acetate buffer. The production of lipase varied widely from strain to strain and seemed to be at a maximum as soon as the cultures had attained the stage of full sporulation. Sodium chloride in concentrations existing in blue cheese did not retard the action of either the lipase or the protease of the mold. These investigators found that the enzymes of *P.*

Received for publication July 22, 1949.

¹ Journal paper no. J1671 of the Iowa Agricultural Experiment Station. Project 895.

roquesforti, added to blue cheese in the form of mycelium, produced cheese of fine quality which was ready for market in 5 mo. as compared to 10 mo. for the control cheese, unhomogenized milk being used for manufacture of the cheese.

METHODS

The methods for the manufacture of the cheese, the determination of fat acidity, the determination of total volatile acidity and the scoring of the cheese are given in a previous paper (6).

The medium used for making the mold-enzyme preparation contained sodium nitrate, 2 g.; monopotassium phosphate, 1 g.; potassium chloride, 0.5 g.; magnesium sulfate, 0.5 g.; ferrous sulfate, 0.01 g.; peptone, 3 g.; butterfat, 3 g.; agar, 5 g.; and water, 1,000 ml. Due to difficulty in sterilization, the butterfat was sterilized separately and added to the semi-solid medium at the time of inoculation. For sterilization, the medium was dispensed in 1-qt. bottles which were only half filled so the butterfat could be emulsified by shaking.

The mold-enzyme preparation was made by inoculating this medium with mold spores, emulsifying the melted butterfat into the medium and placing it in previously sterilized 2,800-ml. Fernbach flasks to a depth not to exceed 1 in. The mold was allowed to grow for 7 days at room temperature (70-75° F.) with shaking at 2-day intervals to break up the surface felt.

RESULTS

The mold-enzyme preparation made with four different mold strains was added to four lots of pasteurized homogenized milk at the rate of 0.55 per cent and the milk was made into blue cheese at once. The total volatile acidities, fat acidities, scores and defect comments for these lots of cheese are presented in table 1.

The total volatile acidities and fat acidities were high for the cheese 4 wk. old. The fat hydrolysis in lots 48 and 51 was excessive at 4 wk., as indicated by the defect comments. At 12 wk. all four lots of cheese had considerable peppery flavor but were too rancid to score for flavor and defect, indicating that too much of the preparation had been used. This is substantiated by the exceedingly high total volatile acidities and fat acidities at 12 wk. Mold strains 4 and 17 appeared to cause more fat hydrolysis than the other strains used.

The preceding trial indicated the possibility for use of the special mold-enzyme preparation if the right mold strain were selected, and if the proper concentration of mold-enzyme preparation were used in the cheese. Mold-enzyme preparations were made with each of five strains of mold and each preparation was added to three vats of milk at the rates of 0.05, 0.10 and 0.25 per cent. Repeat trials were made with two of the mold strains used. The total volatile acidities, scores and defect comments for all the trials are given in table 2.

All lots of cheese made with added mold-enzyme preparation had higher flavor and defect scores at 12 wk. than did corresponding control lots of cheese made without added mold-enzyme preparation. Two of the seven lots of cheese made with the 0.25 per cent concentration of added mold-enzyme preparation were criticized for being excessively sharp. Strains 4, 12 and 6 appeared to effect

TABLE 1
The effect of the addition of 0.55% of mold-enzyme preparation to pasteurized milk

Lot no.	Mold strain no.	Total volatile acidity ^a		Fat acidity ^b		Mold		Score		Comments for cheese 4 wk. old
		4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	Flavor	Defect	
48	4	48.10	107.00	45.13	98.55	4.0	5.0	6.5	7.0	Excessive fatty acids Some ketone, sour, unclean Yeasty, unclean, lacking Some ketone, excessive rancidity
49	12	25.20	38.90	26.90	56.48	5.0	6.5	5.5	4.0	
50	14	22.40	64.00	20.38	50.83	4.0	6.0	4.0	4.0	
51	17	63.00	99.60	48.76	112.63	6.5	4.0	6.0	5.5	

^a Total volatile acidity expressed as ml. 0.1 N acid/200 g. cheese.

^b Fat acidity expressed as ml. 0.1 N acid/10 g. fat.

^c Hydrolysis of fat too extensive to permit accurate scoring at 12 wk.

TABLE 2
The effect of the addition of varying amounts of mold-enzyme preparation of free strains of mold to pasteurized milk.

Lot no.	Mold strain no.	Mold enzyme prep. used	Total volatile acidity ^a		Score				Defect comments for cheese 12 wk. old		
			Mold		Flavor		Defect				
			4 wk.	12 wk.	4 wk.	12 wk.					
		(%)									
92	4	0.00	8.60	28.60	4.0	7.0	3.5	3.0	4.0	2.0	Sour, musty, cheddar
93	4	0.05	9.30	22.30	4.5	7.5	3.0	5.5	7.0	7.0	Sl. nutty
94	4	0.10	9.80	26.00	3.5	4.5	4.5	6.0	6.0	5.0	Sour, sl. unnatural
95	4	0.25	9.50	36.50	3.5	4.5	5.5	7.0	4.5	7.0	Sl. nutty
96	12	0.00	10.00	15.20	6.0	9.0	3.0	4.0	5.5	3.5	Sour, fermented
97	12	0.05	7.50	15.70	8.0	7.5	3.5	6.0	2.0	5.0	Sl. sour, sl. fermented
98	12	0.10	7.50	25.40	8.0	8.0	4.0	6.0	3.0	4.0	Unclean, sour, fermented
99	12	0.25	12.00	32.80	8.0	6.0	5.5	7.5	4.0	7.0	Sl. fermented
100	6	0.00	6.00	22.50	5.0	8.0	4.0	2.5	6.0	2.0	Musty, unnatural
101	6	0.05	12.10	42.50	6.0	7.5	6.5	7.0	7.5	6.5	Unnatural
102	6	0.10	21.00	51.05	4.0	3.5	7.0	7.0	4.5	6.5	Unnatural
103	6	0.25	28.20	78.80	3.5	4.5	7.5	6.5	7.0	5.0	Excessive sharpness
104	13	0.00	9.10	14.50	3.5	5.0	3.0	3.0	2.0	2.0	Musty, sour, unnatural
105	13	0.05	9.70	14.50	7.5	5.0	4.5	5.0	4.5	5.5	Unnatural
106	13	0.10	9.25	15.60	6.5	4.0	4.5	5.0	5.5	6.0	Sl. unnatural
107	13	0.25	8.00	16.40	5.0	4.0	5.5	5.5	7.0	5.0	Unnatural
108	M ₁	0.00	7.10	15.40	4.5	4.0	4.5	4.0	7.5	3.0	Musty, yeasty, sour
109	M ₁	0.05	7.30	17.50	6.0	6.5	5.5	5.0	6.5	5.0	Unnatural, sl. sour, cheddar
110	M ₁	0.10	11.30	17.00	3.5	2.5	5.0	5.5	4.0	5.0	Unnatural, sl. sour, cheddar
111	M ₁	0.25	15.70	29.20	5.5	5.0	6.5	6.0	7.5	5.0	Unnatural, sl. sour, cheddar
140	6	0.00	11.80	17.80	5.5	5.0	4.0	2.5	4.5	2.5	Yeasty, sour, unclean
141	6	0.05	17.40	37.00	6.5	4.5	7.0	7.0	7.0	5.0	Sl. unclean, sl. fermented
142	6	0.10	29.00	57.00	4.5	4.0	7.5	7.5	7.0	7.5	Sl. soapy
143	6	0.25	43.05	80.50	4.0	4.5	6.5	6.5	5.0	6.0	Soapy, excessively sharp
144	M ₁	0.00	8.05	20.50	7.5	5.5	3.0	3.5	4.0	3.0	Fermented, sour, sl. musty
145	M ₁	0.05	10.60	29.00	7.5	5.0	4.5	4.5	4.5	4.0	Sour, sl. fermented
146	M ₁	0.10	12.70	28.20	4.5	3.5	5.5	6.0	6.5	5.5	Sl. sour
147	M ₁	0.25	25.00	50.70	3.0	4.5	6.5	7.0	6.5	6.5	Sl. soapy

^a Total volatile acidity expressed as ml. 0.1 N acid/200 g. cheese.

the most improvement in flavor of the cheese, while strains 13 and M₆ effected the least improvement of the flavor. Strain 6 consistently gave the greatest increase in total volatile acidity of any of those used. It also gave the best flavor, particularly when the total volatile acidity was in the range of 30 to 60 ml. of 0.1N acid per 200 g. cheese.

Reduction of mold scores was observed in many instances when higher levels of mold-enzyme preparation were used, probably because of the known inhibitory effect of free fatty acids upon the development of microorganisms.

DISCUSSION

The use of a mold-enzyme preparation is a method for improving the flavor of blue cheese made from pasteurized homogenized milk. The preparation of the mold-enzyme material and the control necessary for its successful use would be somewhat more involved than the use of an enzyme or a culture in the milk for blue cheese manufacture on a commercial scale. The optimum amount of preparation to use appears to be about 0.25 per cent, which would be 25 lb. per 10,000 lb. vat of cheese. The preparation and incubation of that much material over a 7-day period would involve considerable labor, space and equipment. The mold-enzyme preparation also would serve as the source of mold inoculation of the cheese and, thus, would eliminate the cost of that item.

The reduction of mold growth frequently observed when the effective quantities of the mold-enzyme preparation were used would be undesirable commercially because of the desire of the consumer to purchase a cheese showing good mold growth. No suitable way to overcome this shortcoming has been found.

To be used effectively, the strain of mold would need to be chosen carefully and the amount of preparation to add to the milk would need to be determined for each mold strain under the particular manufacturing and marketing conditions of the individual manufacturing plant.

SUMMARY AND CONCLUSIONS

1. The proper addition of a mold-enzyme preparation to pasteurized homogenized milk increased the total volatile acidity and fat acidity and improved the flavor of blue cheese made from that milk.

2. The use of 0.55 per cent of the preparation in milk caused excessive fat hydrolysis in the cheese in all trials at 12 wk. The optimum amount to use appeared to be about 0.25 per cent for the conditions of these trials.

3. The strains of mold used varied greatly in the amount of hydrolysis which their respective mold-enzyme preparations would cause in cheese.

4. The effective use of this preparation in the improvement of blue cheese made from pasteurized homogenized milk will depend upon careful selection of mold strains to be used and proper adjustment of the amount of preparation to be used in the milk.

REFERENCES

- (1) CURRIE, J. N. Flavor in Roquefort Cheese. *J. Agr. Research*, 2: 1-14. 1914.
- (2) DOX, A. W. The Intracellular Enzymes of *Penicillium* and *Aspergillus* with Special Reference to Those of *Penicillium camemberti*. U. S. Dept. of Agr. Bur. Animal Ind. Bull. 120. 1910.

- (3) FOUTS, E. L. Some Factors Responsible for Variations in the Acid Numbers of the Fat in Cream and in Commercial Butter. J. Dairy Sci., 23: 245-258. 1940.
- (4) KIRSH, D. Factors Influencing the Activity of Fungus Lipase. J. Biol. Chem. 108: 421-430. 1935.
- (5) NAYLOR, N. M., SMITH, L. W., AND COLLINS, H. F. The Esterase and Protease of *Penicillium roqueforti*. Iowa State College J. Sci., 4, 465-471. 1930.
- (6) PARMELEE, C. E., AND NELSON, F. E. The Use of *Candida Lipolytica* Cultures in the Manufacture of Blue Cheese from Pasteurized Homogenized Milk. J. Dairy Sci., 32: 993-1000. 1949.
- (7) THIBODEAU, R., AND MACY, H. Growth and Enzyme Activity of *Penicillium roqueforti*. Minn. Agr. Expt. Sta. Tech. Bull. 152. 1942.

THE RELATION OF CHEMICAL ANALYSES TO THE FLAVOR SCORES OF BLUE CHEESE MADE FROM PASTEURIZED HOMOGENIZED MILK¹

C. E. PARMELEE AND F. E. NELSON

Dairy Industry Section, Iowa Agricultural Experiment Station, Ames

The work of Currie (3) has indicated that the characteristic peppery flavor of blue cheese and the burning effect on the tongue and palate are due to the caproic, caprylic and capric acids and their readily hydrolyzable salts. These accumulate in the cheese as the result of fat hydrolysis by the enzymes of the mold *Penicillium roqueforti*. Hammer and Bryant (5) succeeded in isolating methyl-n-amyl ketone from milk to which n-caprylic acid and mold spores had been added; they believed this compound was responsible for part of the characteristic flavor of blue cheese. Stokoe (14) and Davies (4) have explained the formation of methyl ketones by molds as the second step in an abnormal oxidation of fatty acids. Caprylic acid is the only fatty acid which would yield methyl-n-amyl ketone by this oxidation.

Lane and Hammer (9) found that blue cheese made from pasteurized homogenized milk showed more rapid development of volatile acidity, higher acid values on the fat and more typical flavor than cheese made from raw milk not homogenized; however, it did not ripen as fast as cheese made from raw homogenized milk. In cheese made from raw milk, the volatile acidity increased with increase in the time of ripening and older cheese contained a greater percentage of acids such as caproic, caprylic and capric. The acid values obtained on the cheese fat also increased with extended ripening. These investigators concluded that there was a general relationship between volatile acidities, fat acidities and flavor scores. They also found that the amount of soluble nitrogen in the serum from cheese increased with the age of the cheese but was not related to flavor. The amino nitrogen values and the nitrogen fractions soluble in trichloroacetic acid, ethyl alcohol, and phosphotungstic acid followed about the same pattern as the soluble nitrogen.

METHODS

The cheese was made and scored by the methods given in a previous paper (10). Volatile acidity of the cheese was determined by the method of Lane and Hammer (9). Fat acidity was determined by the method of Breazeale and Bird (2), after the fat was obtained and purified by the method of Lane and Hammer (9).

In making analyses for protein degradation, the cheese was made into a uniform suspension by a modification of the method of Knudsen and Sørensen (7). The cheese suspension was made by emulsifying 25 g. of cheese in 400 ml. of boiling 2.0 per cent sodium citrate solution by agitation in an Eskimo Whiz-mix (model 515JB) for 5 min. at high speed. The mixture was kept alkaline to

Received for publication July 22, 1949.

¹ Journal paper no. J-1670 of the Iowa Agricultural Experiment Station, Project 895.

brom thymol blue by adding alkali when necessary. This suspension was transferred quantitatively to a 500 ml. volumetric flask, cooled to 20° C. and made to volume with distilled water. Preliminary studies of this method of preparing the suspension, using completely dispersible peptone added to milk, indicated the citrate did not influence the nitrogen partition.

The nitrogen fractions soluble and insoluble in phosphotungstic acid were determined by the method of Lane and Hammer (8), with slight modification. Ten ml. of the cheese suspension (equivalent to 0.5 g. of cheese) was treated with a solution containing 50 ml. water, 15 ml. 25 per cent aqueous sulfuric acid and 5 ml. 20 per cent aqueous phosphotungstic acid for 16 to 18 hr. at room temperature. After filtration through a Whatman no. 12, 12.5 cm. fluted filter paper, the precipitate was washed three times with a solution of the same concentration as that used in the precipitation, and the total nitrogen was determined in the filtrate and in the precipitate by the Kjeldahl-Gunning-Arnold method of the Association of Official Agricultural Chemists (1). Copper sulfate was used as the catalyst and the mixed methyl red-methylene blue indicator of Johnson and Green (6) was used in the titration.

The nitrogen fractions soluble and insoluble in trichloroacetic acid were determined by a modification of the method of Lane and Hammer (8) in which cheese suspension equivalent to 0.5 g. of cheese was treated with a solution of 45 ml. water, and 5 ml. 20 per cent aqueous trichloroacetic acid for 16 to 18 hr. at room temperature. Further operations were the same as for the phosphotungstic acid procedure.

The amino nitrogen values for the cheese were determined by placing an amount of the cheese suspension equivalent to 1.0 g. of cheese in a 25 ml. volumetric flask, adding seven drops of glacial acetic acid to precipitate the casein, making to volume, filtering and making a Van Slyke amino nitrogen determination (15) on the filtrate. The values are expressed as milligrams of amino nitrogen per gram of cheese.

The statistical analyses of the data consisted of the calculation according to Snedecor (13) of the equation for the line of linear regression by the method of least squares and of the correlation coefficient (r), and also estimation of the significance of the correlation coefficient from table 7.3 of Snedecor (13).

RESULTS

The relation of fat degradation to flavor score. To learn more of the part which the fatty acids of lower molecular weight contribute to the flavor of the cheese, two series of trials were made in which varying amounts of some of the fatty acids were added to lots of pasteurized homogenized milk which then were made into cheese. In all cases, the fatty acids were added to a 100 g. quantity of melted butterfat, which then was homogenized into 1 qt. of milk and added to the cheese milk before setting with rennet. The same amount of butterfat alone homogenized into milk was added to the control lots. The kinds and amounts of acids added to each lot of cheese, as well as the scores and analyses of each lot of cheese, are presented in table 1.

TABLE 1

The effect of the addition of low molecular weight fatty acids on the score, volatile acidity and fat acidity of pasteurized milk blue cheese

Lot no.	Acid added to 100 g. butterfat ^a					Volatile acidity ^b		Fat acidity ^c	
	Butyric	Caproic	Caprylic	Capric	Lauric	4 wk.	12 wk.	4 wk.	12 wk.
	(g.)	(g.)	(g.)	(g.)	(g.)				
29						8.00	18.40	4.26	20.00
30			2.0			7.45	15.50	4.18	8.45
31			10.0			15.30	18.90	5.25	14.72
52						7.00	13.90	2.65	37.30
53		0.4	0.2	0.4		7.32	12.70	4.00	29.18
54		0.4	0.2	0.4	0.8	6.40	20.60	3.17	29.98
55	0.6	0.4	0.2	0.4	0.8	6.00	14.20	2.74	18.27

Lot no.	Score						Defect comments for cheese 12 wk. old
	Mold		Flavor		Defect		
	4 wk.	12 wk.	4 wk.	12 wk.	4 wk.	12 wk.	
29	6.0	4.5	3.0	4.0	3.0	6.5	Flat, yeasty, nutty
30	5.0	5.5	6.0	6.0	7.0	7.0	Flat, nutty
31	4.0	3.5	6.0	4.5	5.0	4.0	Musty, caprylic acid
52	6.5	5.0	4.5	3.5	5.0	2.5	Nutty, unnatural
53	5.0	6.0	3.5	5.0	4.0	3.5	Sl. nutty, unnatural
54	4.5	7.5	4.5	7.0	5.5	5.5	Sl. nutty, unnatural
55	4.5	7.0	6.0	6.0	8.0	4.5	Sl. nutty, unnatural, unclean

^a The 100 g. melted butterfat were homogenized into 1 qt. of milk and added to the 115 lb. of milk for cheese making.

^b Volatile acidity expressed as ml. 0.1 N acid/200 g. cheese.

^c Fat acidity as ml. 0.1 N acid/10 g. fat.

The cheese with added fatty acids showed definite improvement in flavor score over the control cheese; however, none was typical of good blue cheese, lacking the smoothness and fullness of flavor desired. The fat acidities and total volatile acidities increased considerably from the fourth to the twelfth week. The mold scores at 4 wk. were lowest in the cheese with the largest amounts of added fatty acids, although at 12 wk. there was a tendency for this relationship to be reversed. The fatty acids may be toxic to the mold until the mold is established and has started to utilize the acids in its growth. In the case of lot 31, so much caprylic acid was added that it probably was toxic during the entire 12 wk.

To determine whether a relationship between flavor score and fat acidity could be established with cheese made from pasteurized, homogenized milk, the fat acidities and flavor scores at 12 wk. for lots 29 to 31 and 52 to 55, as shown in table 1 of this paper, and lots 32 to 47 in table 1 of a previous paper (10) were analyzed statistically. The regression equation, degrees of freedom, correlation coefficient and significance of the correlation coefficient are shown in table 2. The data reveal no significant correlation between the fat acidities and the flavor scores of these lots of blue cheese.

The relationship of volatile acidity to flavor score for the lots of cheese to

TABLE 2

Statistical analysis of the relationship at 12 weeks of flavor scores of blue cheese to chemical analyses which indicate decomposition of fats and proteins

Analysis	Regression equation	d.f. ^a	r ^c	Significance of r
Fat acidity	$\hat{Y} = 1.05x + 11.15$	21	0.180	N.S. ^e
Volatile acidity ^a	$\hat{Y} = 4.61x + 3.01$	50	0.688	** ^b
Volatile acidity ^b	$\hat{Y} = 6.83x - 6.33$	29	0.507	**
Amino nitrogen	$\hat{Y} = .399x + 2.73$	29	0.518	**
Trichloroacetic N ^c	$\hat{Y} = .139x + 3.34$	29	0.358	* ¹
Phosphotungstic N ^d	$\hat{Y} = .144x + 1.76$	29	0.478	**

^a *Candida lipolytica* added.

^b Mold enzyme preparation added.

^c Nitrogen fraction soluble in trichloroacetic acid.

^d Nitrogen fraction soluble in phosphotungstic acid.

^e Degrees of freedom.

^f Correlation coefficient.

^g Not significant.

^h **Significant at the 1% level.

ⁱ *Significant at the 5% level.

which *Candida lipolytica* cultures were added (10) is shown in table 2. This relationship for these lots of cheese is highly significant on the basis of the correlation coefficient. The relationship of volatile acidity to flavor score for the lots of cheese to which a mold-enzyme preparation was added (11) is shown in table 2. The relationship is approximately the same as that for the cheese to which *Candida lipolytica* cultures were added.

The relation of protein degradation to flavor score. Amino nitrogen values and nitrogen fractions soluble and insoluble in phosphotungstic acid and in trichloroacetic acid were determined at 12 wk. for the cheese of lots 29 to 31 and 52 to 55 shown in table 1 of this paper and lots 32 to 47 and 56 to 63 shown in tables 1 and 2, respectively, of a previous paper (10). The relationship of amino nitrogen values, nitrogen fractions soluble in trichloroacetic acid and in phosphotungstic acid to flavor scores are shown in table 2. A highly significant correlation between the amino nitrogen values and the flavor scores of the cheese is indicated. The correlation between nitrogen fraction soluble in trichloroacetic acid and flavor score was significant at the 5 per cent level of probability. The correlation between nitrogen fraction soluble in phosphotungstic acid and flavor score is highly significant at the 1 per cent level of probability.

DISCUSSION

The data presented indicate no correlation of fat acidity to flavor score among the lots of cheese upon which fat acidities were determined. As the samples of fat were obtained with great difficulty, they possibly were not representative of all the fat in the cheese. The results in table 2 show a highly significant correlation of volatile acidity and flavor score for the lots of cheese made from pasteurized homogenized milk to which *C. lipolytica* cultures or mold enzyme prep-

arations had been added. In general, the cheese with highest flavor scores had volatile acidity values in the range of 30 to 55 ml. of 0.1 *N* acid per 200 g. of cheese. This range agrees well with that published by Peters and Nelson (12).

The data on protein degradation, although obtained on only a limited number of samples of cheese, indicate that protein degradation by the criteria employed is correlated with flavor development in blue cheese made from pasteurized, homogenized milk. This is contrary to the findings of Lane and Hammer (9) for cheese made from raw, homogenized milk. Possibly this is an associative rather than a direct relationship, since the flavor and aroma constituents which have been identified or suggested all have been derived from fat rather than from protein. The role which protein degradation plays in determining the desirable body characteristics of the cheese is considerable and contributes much to the consumer acceptance of the cheese. Protein degradation products undoubtedly combine with some of the fat degradation products or reduce the flavor intensity in other ways, with the result that some of the rawness of fat degradation products becomes integrated into a well balanced flavor, acceptable to the consumer. The present studies do not eliminate the possibility that cheese showing results of considerable proteolytic action but deficient in flavor could be produced under other experimental conditions.

The work with the addition of free fatty acids, while limited in scope, indicates definitely that these acids do contribute to the flavor of blue cheese. Their presence in excessive amounts, particularly when mold growth is restricted by their presence, results in cheese which has an undesirable rawness of flavor, presumably due to a disturbed balance in the mechanism of flavor production. The exact ratios of the various acids and the absolute amounts which should be used for optimum flavor production were not determined, since this portion of the study was designed only to obtain some direct evidence on the role of lower fatty acids in flavor development before study of microbial means of obtaining increased fat degradation in blue cheese was initiated. Under present rulings of regulatory officials, addition of the free fatty acids would not be permitted, even if the details of the procedure were to be worked out in such a way as to give a highly desirable type of flavor fortification to the cheese. Reliance on any such fortification also might prove extremely undesirable because flavor development might then precede proper body development, resulting in a product of reduced consumer acceptance.

SUMMARY AND CONCLUSIONS

1. The addition of low molecular weight fatty acids to pasteurized homogenized milk improved the flavor of blue cheese made from that milk. Such cheese lacked the fullness of flavor desired in typical blue cheese.
2. No correlation was found between fat acidities and flavor scores of blue cheese made from pasteurized homogenized milk.
3. A highly significant correlation was found between volatile acidity and flavor score of blue cheese made from pasteurized homogenized milk to which lipases or organisms producing lipases had been added. In general, the cheese

with the highest flavor scores had volatile acidities in the range of 30 to 55 ml. of 0.1 *N* acid per 200 g. of cheese.

4. The amino nitrogen values were correlated at a highly significant level with the flavor scores of blue cheese made from pasteurized homogenized milk.

5. Nitrogen fractions soluble in trichloroacetic acid and nitrogen fractions soluble in phosphotungstic acid were correlated significantly and highly significantly, respectively, to the flavor scores of blue cheese made from pasteurized homogenized milk.

REFERENCES

- (1) Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*. 6th ed. Washington, D. C. Association of Official Agricultural Chemists. 1945.
- (2) BEEZEALE, D. F., AND BIRD, E. W. A Study of Methods for the Determination of Acidity in Butterfat. *J. Dairy Sci.*, 21: 335-344. 1938.
- (3) CURRIE, J. N. Flavor in Roquefort Cheese. *J. Agr. Research*, 2: 1-14. 1914.
- (4) DAVIES, W. L. The Deterioration of Fats and the Development of Rancidity. *Ind. Chemist*, 4: 269-272. 1928.
- (5) HAMMER, B. W., AND BRYANT, H. W. A Flavor Constituent of Blue Cheese (Roquefort Type). *Iowa State College J. Sci.*, 11: 281-285. 1937.
- (6) JOHNSON, A. H., AND GREEN, J. R. Modified Methyl Red and Sodium Alizarin Sulfonate Indicators. *Ind. Eng. Chem., Anal. Ed.*, 2: 2-4. 1930.
- (7) KNUDSEN, SØNCKE, AND SØRENSEN, A. Untersuchungen von Käse mit Hilfe einer Natriumcitrat unthaltender Lösung. I. Bestimmung der Bakterienmenge in Käse. *Kong. veterin.-og landsbohejskole, Aarskr.*, 1942: 11-15. 1942.
- (8) LANE, C. B., AND HAMMER, B. W. Effect of Pasteurizing the Milk on the Nitrogenous Decomposition in Cheddar Cheese. *Iowa Agr. Expt. Sta. Research Bull.* 183. 1935.
- (9) LANE, C. B., AND HAMMER, B. W. Some factors Affecting the Ripening of Blue (Roquefort Type) Cheese. *Iowa Agr. Expt. Sta. Research Bull.* 237. 1938.
- (10) PARMELEE, C. E., AND NELSON, F. E. The Use of *Candida lipolytica* Cultures in the Manufacture of Blue Cheese from Pasteurized Homogenized Milk. *J. Dairy Sci.*, 32: 993-1000. 1949.
- (11) PARMELEE, C. E., AND NELSON, F. E. The Use of a Mold-Enzyme Preparation in the Manufacture of Blue Cheese from Pasteurized Homogenized Milk. *J. Dairy Sci.*, 32: 1001-1006. 1949.
- (12) PETERS, I. L., AND NELSON, F. E. The influence of *Mycotorula lipolytica* Lipase upon the Ripening of Blue Cheese Made from Pasteurized Homogenized Milk. *J. Dairy Sci.*, 31: 611-618. 1948.
- (13) SNEDECOR, G. W. *Statistical Methods*. 4th ed. Pp. 103-168. Iowa State College Press, Inc., Ames, Iowa. 1946.
- (14) STOKOE, W. N. The Rancidity of Coconut Oil Produced by Mould Action. *Biochem. J.*, 22: 80-93. 1928.
- (15) VAN SLYKE, D. D. A Method for Quantitative Determination of Aliphatic Amino Groups. *J. Biol. Chem.*, 9: 185-204. 1911.

VITAL STUDIES ON BULL SEMEN USING TRIPHENYLTETRAZOLIUM CHLORIDE¹

JOHN P. MIXNER

New Jersey Agricultural Experiment Station, Sussex

Triphenyltetrazolium chloride (TTC) is colorless in solution but forms an insoluble red compound, triphenyl formazen, upon reduction (3). This property of TTC has lent itself to various physiological studies with living tissues involving reducing enzymes. This chemical has been used as a test reagent for seed germination studies (3), to demonstrate reducing enzyme systems in neoplasms and other living mammalian tissues (5), as a vital dye for stem tissues of plants (6) and for a wide variety of living tissues including bull sperm and the serum of bull sperm (3). The latter observation prompted these studies on the possible application of TTC in vital studies with bull semen and spermatozoa. Of particular importance would be its use as a stain to differentiate live and dead sperm (2) and as an indicator of sperm viability, much as the methylene blue reduction test is used (1).

MATERIALS AND METHODS

The semen used in these studies was collected with an artificial vagina from dairy bulls in the stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex. The semen diluter (4) used in toxicity trials was composed of equal part by volume of egg yolk and a 3.6 per cent solution of sodium citrate dihydrate, with sulfanilamide added at the rate of 3 mg. per ml. of complete diluter.

The duration of motility of spermatozoa incubated at 46.5° C. in a water bath was used as the measure of toxicity of the TTC. This duration of motility was expressed as a percentage of that of a control semen dilution containing no TTC.

To obtain sperm-free seminal plasma, the semen was centrifuged to throw down most of the spermatozoa and then the decanted plasma was filtered with a micro-Boerner centrifuge filter. This gives a seminal plasma that is absolutely free of sperm.

The amount of red color developed in any given trial with TTC was rated on a scale from 5 to 0, 5 being the highest and 0, no color. The best color development was an extremely brilliant red which started to develop within 5 min. after the addition of the TTC.

RESULTS AND DISCUSSION

To determine the toxicity of TTC, three separate semen samples were diluted with the egg yolk citrate-sulfonamide diluter at the rate of one part of semen to nine parts of diluter. TTC was added to 1 ml. portions of these diluted semen samples according to the schedule shown in table 1. The data for the three sam-

Received for publication August 1, 1949.

¹ Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Dairy Industry.

TABLE 1
Toxicity of triphenyltetrazolium chloride

Dosage TTC/ml.	Av. duration of motility as % of controls	Av. color rating
(mg.)		
0 (Control)	100	0
0.001	94.1	0
0.01	86.8	0
0.1	75.0	1
1.0	33.8	2
5.0	8.8	3
10.0	2.8	2
20.0	<2.0	1

ples were averaged and the results are presented in the table. These data show that TTC is extremely toxic to bull spermatozoa. At the level of 5 mg. TTC per ml. the duration of motility of spermatozoa was 8.8 per cent of that of the controls. However, the red color development was greatest at this level. In subsequent experiments the level of 5 mg. of TTC per ml. of semen or diluter was used in rating color development or reduction of TTC.

That the reduction of TTC is proportional to the concentration of semen in the diluter is shown by the data in table 2. Several dilutions of a semen sample

TABLE 2
Concentration of semen in relation to reduction of TTC

Semen dilution	Dosage TTC (mg./ml.)	Color rating
1 ml. semen	0	0
1 ml. semen	5	5
1 ml. 1:4 dilution of semen	5	4
1 ml. 1:9 " " "	5	3
1 ml. 1:99 " " "	5	0
1 ml. 1:999 " " "	5	0

were reacted with TTC and color reactions were rated at the end of 1 hr. incubation at 46.5° C.

Semen smears for microscopic examination were made from the tube with the highest color reaction; the spermatozoa were unstained or, at best, had an extremely light red stain.

The question arises as to whether the live spermatozoa, dead spermatozoa or seminal plasma may be responsible for the reduction of the TTC. In this connection several types of semen preparations have been reacted with TTC for 3 hr. at 46.5° C. at the rate of 5 mg. of TTC per ml. The data are presented in table 3. These trials seem to indicate that the reduction of TTC can not be caused by seminal plasma alone but rather by either live or recently killed spermatozoa. However, live spermatozoa gave a much more intense color reaction than did recently killed spermatozoa.

The high toxicity of TTC in semen, together with its extremely poor capacity

TABLE 3
Color reactions of various semen preparations

Sample no.	Description of sample	Color rating
1	1 ml. fresh semen	5
2	1 ml. plasma from fresh semen	0
3	1 ml. fresh semen—heat (46.5° C.) and cold (0° C.) shocked repeatedly to kill spermatozoa	2
4	1 ml. plasma from no. 3	0
5	1 ml. semen + 0.2 ml. toluene, incubated for 3 hr. to kill spermatozoa	2
6	1 ml. plasma from no. 5	0
7	1 ml. fresh semen heated to 82° C. for 20 min.	0

for staining spermatozoa, seem to preclude its use either as a vital stain for spermatozoa or as a general indicator of spermatozoa vitality.

SUMMARY

Triphenyltetrazolium chloride is readily reduced to triphenylformazen, an insoluble red compound, by fresh dairy bull semen. Semen in which the spermatozoa have been killed by heat- and cold-shocking or by treatment with toluene also has the ability to reduce the compound, but to a lesser degree. The heating of semen to 82° C. for 20 min. destroyed its reducing ability. Seminal plasma exhibited no ability to reduce the tetrazolium.

As judged by the effects of tetrazolium on the length of time which spermatozoa will maintain motility when incubated at 46.5° C., tetrazolium is very toxic. This together with its inability to stain spermatozoa adequately in its reduced state, precludes its use as a vital stain for spermatozoa or in measuring spermatozoa viability.

REFERENCES

- (1) BECK, G. H., AND SALISBURY, G. W. Rapid Methods for Estimating the Quality of Bull Semen. *J. Dairy Sci.*, **26**: 483-494. 1943.
- (2) LASLEY, J. F., EASLEY, G. T., AND MCKENZIE, F. F. A Staining Method for the Differentiation of Live and Dead Spermatozoa. *Anat. Record*, **82**: 167-174. 1942.
- (3) MATTSON, A. M., JENSEN, C. O., AND DUTCHER, R. A. Triphenyltetrazolium Chloride as a Dye for Vital Tissues. *Science*, **106**: 294-295. 1947.
- (4) SALISBURY, G. W. AND KNOTT, C. B. The Effect of Sulfanilamide in the Diluent upon Fertility of Bull Semen. *J. Dairy Sci.*, **30**: 361-369. 1947.
- (5) STRAUS, F. H., CHERONIS, N. D., AND STRAUS, E. Demonstration of Reducing Enzyme Systems in Neoplasma and Living Mammalian Tissues by Triphenyltetrazolium Chloride. *Science*, **108**: 113-115. 1948.
- (6) WAUGH, T. D. Staining of the Stem Cells of Plants by Triphenyltetrazolium Chloride. *Science*, **107**: 275. 1948.

THE INFLUENCE OF PASTURE AND EARLY RUMEN DEVELOPMENT ON THE CHANGES IN THE PLASMA CAROTENOIDS, VITAMIN A AND ASCORBIC ACID AND THE LIVER STORAGE OF CAROTENOIDS AND VITAMIN A OF YOUNG DAIRY CALVES

J. W. HIBBS AND W. D. POUNDEN

Department of Dairy Industry, Ohio Agricultural Experiment Station, Wooster

The favorable results obtained from feeding relatively large amounts of good quality hay, coupled with rumen inoculations, as a means of initiating early rumen function and meeting some of the vitamin needs of young calves, suggested the feasibility of utilizing pasture, when available, in young calf feeding.

In an earlier communication (2), the changes in the blood plasma carotenoids, vitamin A and ascorbic acid during the first 6 wk. in calves fed milk and alfalfa hay with and without grain concentrates and with and without rumen inoculations with cud material from older cattle were presented. Calves fed on a ration consisting of whole milk and alfalfa hay had a much higher blood plasma carotenoid level than calves fed the same ration plus grain concentrates free choice. Little, if any, difference in plasma vitamin A was shown between the two groups. No effect from rumen inoculations could be detected in either the plasma carotenoids or vitamin A. However, a higher, more uniform level of ascorbic acid was maintained in inoculated calves fed only milk and alfalfa hay than in the uninoculated calves fed the same ration.

In accompanying reports (5, 6), the influence of the ration, including various proportions of grain concentrates to roughage ingestion, on the establishment of certain rumen microorganisms was shown.

This report presents the results obtained from feeding young calves on pasture with variations in supplemental hay and grain feeding and rumen inoculations. The changes in the blood plasma carotenoids, vitamin A and ascorbic acid, liver storage of carotenoids and vitamin A and the gain in body weight are included. The influence of pasture and rumen inoculations on the establishment of certain rumen microorganisms in the same calves is presented in an accompanying paper (7).

EXPERIMENTAL

Plasma carotenoids and vitamin A were determined by the procedure of Kimble (3), plasma ascorbic acid according to Mindlin and Butler (4) and liver carotenoids and vitamin A by an adaptation of the method of Guilbert and Hart (1).

Experiment with young calves. Fifteen calves of both the Jersey and Holstein breeds were assigned at birth to one of five groups. The calves in groups

Received for publication Aug. 18, 1949

I and III were inoculated on the fifth, tenth, fifteenth and twenty-first days of age with cud material from older cattle (5) which were eating pasture. The calves in groups II, IV and V were not inoculated.

All the calves were allowed to nurse their dams for the first 3 days and then were fed whole milk at the rate of 0.9 lb. per 10 lb. of body weight, based on the birth weight. Beginning on the fourth day of age, the calves in the first four groups were tethered out during the day on a lawn type (bluegrass, white clover) pasture. The stakes were moved periodically so as to provide fresh grazing. Some variation in the quality of the pasture resulted from weather conditions, but for the most part, it was of good quality. The inoculated calves were kept separate from the uninoculated calves, both while on the pasture and when in the barn at night. One calf in each of the first four groups also was offered clover-timothy hay (second cutting) free choice while in the barn at night.

The three calves in group V were not given access to pasture. They were given clover-timothy hay (second-cutting) and a 14 per cent simple grain mixture free choice, beginning at 14 days. Groups I and II were not fed any grain supplement in addition to pasture and milk, while groups III and IV were given the 14 per cent simple grain mixture free choice, throughout the experiment. The experiment was terminated at the end of the sixth week.

Data showing the feed consumption and body weight gains are presented in table 1. The calves in all groups were bled as nearly as possible on the fourth and seventh days and weekly thereafter through the sixth week, and the samples were analyzed for carotenoids, vitamin A and ascorbic acid. Ten of the calves were slaughtered at 6 wk. of age and the livers were analyzed for carotenoids and vitamin A. The results of the blood and liver analyses for carotenoids are shown in table 2, those for vitamin A in table 3, and those for ascorbic acid in table 4.

Experiment with older calves. Blood plasma carotenoids, vitamin A and ascorbic acid analyses also were made periodically on five of the six older calves which are mentioned in the accompanying paper (7), after they were turned out to pasture. The five calves ranged in age from 62 to 96 days of age (average 71 days) on June 7, 1948, when they were put on a permanent bluegrass, white clover pasture.

Three of the calves had had their rumens inoculated artificially during the first 3 wk. after birth and two of them had not been inoculated. One of the latter two had picked up a partial inoculation in a natural manner. One of the three inoculated calves was given a fresh inoculation from a cow on pasture just before the calf was put on pasture. The changes in plasma carotenoids, vitamin A and ascorbic acid during the 5 wk. following the change from dry feeds to pasture are shown in table 5.

RESULTS AND DISCUSSION

Experiment with young calves. As shown in table 1, the calves that were offered hay ate a small amount in addition to the pasture. Most of the calves

TABLE 1
Feed consumption and body weight gains during the first 6 weeks of calves started on pasture

Group no.	Calf no.	Feed consumption								Birth wt.	Body wt. gains at		Remarks
		Whole milk ^a				Hay ^b		Grain ^c			21 d.	42 d.	
		Age (days)		Age (days)		Age (days)		Age (days)					
		4-21	22-42	4-21	22-42	4-21	22-42	4-21	22-42				
		(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(d.)	(%)	(%)	
I	1H ♀	137	168	0	0	0	0	0	0	39	12.0	28.3	Inoculated. No grain fed.
	2J ♀	90	105	0	0	0	0	0	36	6.9	32.8		
	8H ♀	144	168	0	0	0	0	0	38	14.0	39.6		
	Av.	127	147	—	—	—	—	—	38	11.0	33.6		
II	3H ♂	162	189	0	0	0	0	0	39	14.9	42.5	Not inoculated. No grain fed.	
	15J ♀	90	105	0	0	0	0	0	39	5.0	16.7		
	9H ♂	162	189	0.5	10.0	0	0	0	37	12.0	40.0		
	Av.	138	161	—	—	—	—	—	38	10.6	33.0		
III	4J ♀	90	105	0	0	1.0	9.5	2.5	39	16.4	40.0	Inoculated. Fed grain.	
	5H ♂	162	158	0	0	5.0	8.5	5.5	35	19.3	47.7		
	11J ♀	82	105	6.0	2.5	5.0	5.5	5.5	39	4.2	37.5		
	Av.	111	123	—	—	3.7	7.8	7.8	38	13.3	41.7		
IV	6J ♂	90	105	0	0	1.0	7.0	7.0	39	16.1	40.3	Not inoculated. Fed grain.	
	14H ♀	133	147	0	0	7.5	11.0	11.0	40	10.2	39.7		
	12J ♀	80	105	4.0	3.0	5.5	10.0	10.0	38	12.5	45.8		
	Av.	101	119	—	—	4.7	9.3	9.3	39	12.9	41.9		
V	7H ♂	144	168	1.0	9.0	1.0	9.0	9.0	0	13.8	58.6	Controls. No pasture. Fed grain and hay after 2 wk.	
	10J ♂	86	92	2.0	3.5	1.5	3.5	3.5	0	2.2	22.7		
	13H ♂	118	168	0.5	4.0	0.5	6.0	6.0	0	5.4	26.9		
	Av.	116	143	1.2	5.5	1.0	6.2	6.2	0	7.1	36.1		

^a Nursed dam first 3 d.
^b Second cutting clover-timothy.
^c 14% protein herd ration.
^d Green lawn (Blue grass-white clover).

^a Ad libitum. No record kept of wt.

^a Nursed dam first 3 d.

^b Second cutting clover-timothy.

^c 14% protein herd ration.

^d Green lawn (Blue grass-white clover).

^e Ad libitum. No record kept of wt.

TABLE 2
Changes in the blood plasma carotenoids during the first 6 weeks and total liver carotenoids at 6 weeks of age in calves started on pasture

Group no.	Calf no.	Age of animals (days)						Total liver carotenoids at 42 d.	Remarks
		4	7	14	21	28	35		
		(γ carotenoids/100 ml. plasma)						(γ)	
I	1H ♀	52.0	34.8		35.6		164.0	151.0	Inoculated. No grain fed.
	2J ♀		55.3	79.5	166.0	204.0	269.0	318.0	
	8H ♀ ^b	75.3		65.8	37.3	38.8	134.0	186.0	
	Av.				79.6		189.0	218.3	
II	3H ♂	34.9		87.7	78.4	106.0	138.0	326.0	Not inoculated. No grain fed.
	15J ♀	41.2		24.0	147.0	184.0	89.5 ^a	169.0	
	9H ♂ ^b	35.6	38.8	33.3	64.0	112.0	150.0	200.0	
	Av.	37.2		48.3	96.5	134.0	125.8	231.7	
III	4J ♀	117.0		81.0	105.0	102.0	259.0	410.0	Inoculated. Fed grain.
	5H ♂	42.0		41.2	29.4	112.0	191.0	287.0	
	11J ♀ ^b		38.8	19.5	26.3	116.0	221.0	227.0	
	Av.			47.3	53.6	110.0	223.7	308.0	
IV	6J ♂	129.0	115.0	93.5	83.0	134.0	334.0	199.0 ^a	Not inoculated. Fed grain.
	14H ♀	34.0		34.9	27.9	26.3	83.0	193.0	
	12J ♀ ^b		23.2	9.9	75.5	244.0	363.0	392.0	
	Av.			46.1	62.1	134.8	260.0	261.0	
V	7H ♂	20.2	25.4	28.6	36.4	25.6	27.9	29.5	Controls. No pasture.
	10J ♀	47.7		24.6	9.9	7.1	11.4	17.3	
	13H ♂		15.8	9.9	12.9	23.2	16.5	15.8	Fed grain and hay after 2 wk.
	Av.			21.0	19.7	18.6	18.6	20.9	

^a Diarrhea when bled.

^b Fed supplemental hay in the barn at night (Clover-timothy, second cutting).

TABLE 3
Changes in the blood plasma vitamin A during the first 6 weeks and total liver vitamin A at 6 weeks of age in calves started on pasture

Group no.	Calf no.	Age of animals (days)					Total liver vitamin A at 42 d.	Remarks	
		4	7	14	21	28			35
		(γ vitamin A/100 ml. plasma)					(γ)		
I	1H ♀	12.0	14.2	14.8	14.8	15.7	14.9	13.5	Inoculated. No grain fed.
	2J ♀		20.4	18.9	14.8	15.7	17.5	9.9	
	8H ♀ ^b	23.8		16.7	9.8	10.3	8.0	8.9	
	Av.				13.1		13.5	10.8	
II	3H ♂	18.7		10.6	17.4	14.6	12.0	11.7	Not inoculated. No grain fed.
	15J ♀	26.2		21.7	18.4	16.5	13.3 ^a	14.6	
	9H ♂ ^b	14.9	15.4	14.3	9.8	9.6	9.4	20.4	
	Av.	19.9		15.5	15.2	13.6	11.6	15.6	
III	4J ♀	24.4		15.4	10.8	9.2	8.9	14.9	Inoculated. Fed grain.
	5H ♂	24.0		13.1	12.8	13.7	13.2	15.1	
	11J ♀ ^b		26.2	13.7	10.5	10.4	13.2	13.9	
	Av.			14.1	11.4	11.1	11.8	14.6	
IV	6J ♀	26.6	19.7	12.6	13.3	14.9	15.4	12.7 ^a	Not inoculated. Fed grain.
	14H ♀	17.2		18.7	10.3	9.7	11.5	13.1	
	12J ♀ ^b		12.8	15.8	14.5	18.7	15.3	15.2	
	Av.			15.7	12.7	14.4	14.1	13.7	
V	7H ♂	17.5	20.4	16.1	11.2	12.6	10.7	10.6	Controls. No pasture. Fed grain and hay after 2 wk.
	10J ♂	15.6		12.0	7.3	3.1	4.5	4.8	
	13H ♂		9.8	8.0	8.2	9.2	7.3	8.3	
	Av.			12.0	8.9	8.3	7.5	7.9	

^a Diarrhea when bled.

^b Fed supplemental hay in the barn at night (Clover-timothy, second cutting).

TABLE 4
Changes in the blood plasma ascorbic acid during the first 6 weeks in calves started on pasture

Group no.	Calf no.	Age of animals (days)						Remarks
		4	7	14	21	28	35	
		(mg. ascorbic acid/100 ml. plasma)						
I	1H ♀	0.40	0.33		0.38		0.31	Inoculated. No grain fed.
	2J ♀		0.48		0.38	0.40	0.41	
	8H ♀ ^b	0.55		0.52	0.25	0.62	0.29	
	Av.				0.34		0.34	
II	3H ♂	0.45		0.53	0.39	0.16	0.41	Not inoculated. No grain fed.
	15J ♀	0.59		0.77	0.73	0.51	0.64 ^a	
	9H ♂ ^b	0.74	0.78	0.45	0.38	0.56	0.54	
	Av.	0.59		0.58	0.50	0.41	0.53	
III	4J ♀	0.67		0.52	0.36	0.30	0.51	Inoculated. Fed grain.
	5H ♂	0.58		0.36	0.29	0.45	0.54	
	11J ♀ ^b		0.82	0.36	0.49	0.93	0.64	
	Av.			0.41	0.38	0.56	0.56	
IV	6J ♂	0.69	0.47	0.59	0.34	0.59	0.51	Not inoculated. Fed grain.
	14H ♀	0.60		0.55	0.31	0.31	0.48	
	12J ♀ ^b		0.60	0.35	0.42	0.77	0.57	
	Av.			0.50	0.36		0.53	
V	7H ♂	0.90	0.41	0.30	0.20	0.54	0.54	Controls. No pasture.
	10J ♂	0.41		0.22	0.30	0.15	0.25	Fed grain and hay after 2 wk.
	13H ♂		0.39	0.25	0.60	0.81	0.19	
	Av.			0.25	0.37	0.50	0.33	

^a Diarrhea when bled.

^b Fed supplemental hay in the barn at night (Clover-timothy, second cutting).

TABLE 5

Changes in the blood plasma carotenoids, vitamin A, and ascorbic acid of calves given access to pasture at approximately 10 weeks of age

Calf no.	Before pasture ^a	Days after access to pasture ^b					Remarks (Before pasture)
		14	23	30	38	52	
Plasma carotenoids (γ /100 ml.)							
16J ♂	42.8	368	416 ^c	437	316	280	Inoculated
17J ♀	83.0	384	292 ^c	399	398	305	Inoculated
18H ♂ ^b	38.8	264	292	312	276	190	Inoculated
19H ♀	11.4	366	181	232	255	221	Partial natural inoculation
20H ♀	37.2	384	463	418	307	206	Not inoculated
Av.	42.6	353	329	360	310	240	
Plasma vitamin A (γ /100 ml.)							
16J ♂	7.5	12.0	13.5 ^c	12.2	15.0	20.8	Inoculated
17J ♀	8.2	21.1	21.6 ^c	11.9	24.8	24.4	Inoculated
18H ♂ ^d	8.2	11.7	13.9	10.8	9.9	14.8	Inoculated
19H ♀	12.8	21.4	21.2	22.0	22.3	19.4	Partial natural inoculation
20H ♀	6.9	16.6	20.2	16.4	15.5	17.0	Not inoculated
Av.	8.7	16.6	18.1	14.7	17.5	19.3	
Plasma ascorbic acid (mg./100 ml.)							
16J ♂	.37	.55	.47 ^c	.44	.41	.37	Inoculated
17J ♀	.60	.61	.23 ^c	.35	.42	.46	Inoculated
18H ♂ ^d	.64	.70	.54	.52	.55	.45	Inoculated
19H ♀	.32	.70	.48	.37	.42	.36	Partial natural inoculation
20H ♀	.68	.73	.78	.47	.47	.44	Not inoculated
Av.	.52	.66	.50	.43	.45	.42	

^a Av. age 50 d.

^b Av. age 71 d. at beginning of pasture June 7, 1948.

^c Fed 1.5 lb. of grain daily after July 2, 1948.

^d Freshly inoculated just before pasture period from cow eating pasture.

made satisfactory gains in body weight; however, considerable variation was observed. The calves fed on pasture and milk only (groups I and II) made an average increase in weight from birth to 6 wk. of 33.3 per cent while the calves that were fed grain in addition to pasture and milk (groups III and IV) averaged 41.8 per cent increase. No difference could be detected between the calves which were inoculated and those that were not, so far as their gains in body weight were concerned. This was true in both the grain-fed and no grain-fed groups.

Blood plasma carotenoids of the pasture calves (groups I, II, III and IV) increased rapidly reaching an extremely high but variable level (average 255 γ per 100 ml.) at 6 wk. of age. The calves fed grain concentrates (groups III and IV) increased at a somewhat slower rate during the first 4 wk., but during the fifth and sixth wk. increased much more rapidly than the no grain groups (I and II). Much of this apparent difference was due to the extremely high levels attained by two calves, 4J and 12J.

No difference was found between the inoculated and uninoculated calves so far as their plasma carotenoids were concerned, which is in agreement with the observations made previously (2) when hay was fed instead of pasture.

The control calves (group V) fed in the barn did not show any increase in plasma carotenoids during the entire 6-wk. period. As they were fed the same milk as the calves in the other groups, it is apparent that the increases in carotenoids in the other groups were due principally to utilization of carotenoids from the pasture grass.

Liver storage of carotenoids was approximately seven times higher, on the average, in groups I, II, III and IV than in group V. While the liver storage of carotenoids is somewhat variable, no marked differences among the first four groups can be seen. Unfortunately, liver storage data were not available in group I.

The changes in the plasma vitamin A, although quite variable indicated no marked difference among the pasture fed calves (groups I, II, III and IV). The plasma vitamin A levels in these calves were consistently much higher than those in the control group V. The vitamin A liver storage data do not show any clear cut difference among the pasture groups II, III and IV. No data were available in group I. The average liver storage of vitamin A of the pasture-fed groups was, however, more than twice that of the barn-fed calves.

The changes in the plasma ascorbic acid were variable, but were mostly in the normal range. No particular significance can be attached to the trends in the data. The calves in control group V at 14 days of age had an average level of 0.25 mg. per 100 ml. which is extremely low as compared to the other four groups.

Experiment with older calves. When the five older calves (average age 71 days) were turned out to pasture a marked increase in both plasma carotenoids and vitamin A occurred (table 5). Within 2 wk. the plasma carotenoids had increased more than eight times the pre-pasture level and the vitamin A nearly doubled. These high levels were maintained throughout the pasture period except for a decline in the carotenoids at the end, which probably was due to dry weather and maturing of the bluegrass. The accompanying rise in plasma vitamin A is another example of the increase in plasma vitamin A often observed concurrent with the fall from a high plasma carotene level to a lower level, as previously discussed (2).

No marked changes in the plasma ascorbic acid resulted from access to pasture, except that a temporary average increase was noted just after the change from dry feed to pasture.

SUMMARY AND CONCLUSIONS

An experiment was conducted to measure the influence of pasture and early rumen development on the performance of calves and as a means of meeting some of their vitamin needs. Twelve calves, one-half of which were rumen inoculated with cud material from older cattle, were tethered during the day on a lawn-type bluegrass-white clover pasture beginning at 4 days of age. One-half of the calves in both groups were fed a 14 per cent simple grain mixture free choice while on pasture. The pasture calves were compared to three calves fed in the barn on dry feeds. Milk feeding of all calves was limited to 0.9 lb. per 10 lb. of body weight at birth.

The calves on pasture were able to utilize the nutrients from the grass, as indicated by their high blood plasma and liver carotenoid and vitamin A levels plus satisfactory growth and appearance. The calves fed grain in addition to pasture increased in body weight more rapidly than those that did not receive grain. The plasma carotenoids of the pasture-fed calves averaged 255 γ per 100 ml. at 42 days of age. The average plasma ascorbic acid level at 14 days of age was also higher in the pasture-fed calves than in those that did not receive pasture. Otherwise, no marked differences were observed in the plasma ascorbic acid among the groups.

Rumen inoculations were not shown to affect the blood or liver vitamin levels which were observed, even though the inoculations and variations in the feed resulted in marked differences in the rumen microorganism picture (7).

Data also are presented showing the changes in plasma carotenoids, vitamin A and ascorbic acid before and after turning five older calves out to pasture. These calves had been fed in the barn, three with and two without rumen inoculation, prior to the pasture period.

Based on these findings, plus those presented in an accompanying paper (7), it is concluded that good pasture grass, when available, can be utilized by calves, even at an early age, as an effective means of meeting some of their vitamin needs and as a source of other nutrients.

The authors wish to acknowledge the assistance of John Tate, Miss Barbara L. Carson and C. E. Knoop in conducting this investigation.

REFERENCES

- (1) GUILBERT, H. R., AND HART, G. H. Storage of Vitamin A in Cattle. *J. Nutr.*, 8: 25-44. 1934.
- (2) HIBBS, J. W. AND POUNDEN, W. D. The Influence of the Ration and Early Rumen Development on the Changes in the Plasma Carotenoids, Vitamin A and Ascorbic Acid of Young Dairy Calves. *J. Dairy Sci.*, 31 (12): 1055-1061. 1948.
- (3) KIMBLE, M. S. The Photoelectric Determination of Vitamin A and Carotene in Human Plasma. *J. Lab. Clin. Med.*, 24: 1055-1065. 1939.
- (4) MINDLIN, R. L. AND BUTLER, A. M. The Determination of Ascorbic Acid in Plasma: A Macromethod and a Micromethod. *J. Biol. Chem.*, 122: 673-686. 1938.
- (5) POUNDEN, W. D. AND HIBBS, J. W. The Influence of the Ration and Rumen Inoculation on the Establishment of Certain Microorganisms in the Rumens of Young Calves. *J. Dairy Sci.*, 31 (12): 1041-1050. 1948.
- (6) POUNDEN, W. D. AND HIBBS, J. W. The Influence of the Ratio of Grain to Hay in the Ration of Dairy Calves on Certain Rumen Microorganisms. *J. Dairy Sci.*, 31 (12): 1051-1054. 1948.
- (7) POUNDEN, W. D. AND HIBBS, J. W. The Influence of Pasture and Rumen Inoculation on the Establishment of Certain Microorganisms in the Rumens of Young Calves. *J. Dairy Sci.*, 32 (12): 1025-1031. 1949.

THE INFLUENCE OF PASTURE AND RUMEN INOCULATION ON THE ESTABLISHMENT OF CERTAIN MICROORGANISMS IN THE RUMEN OF YOUNG DAIRY CALVES

W. D. POUNDEN AND J. W. HILBBS

Ohio Agricultural Experiment Station, Wooster, Ohio

The use of certain rumen microorganisms as indicators of the presence or absence of characteristic flora in young calves was described in a previous report concerning investigations of rumen development in these animals (4). The particular bacteria chosen for this purpose were observed quite regularly in samples from mature stock consuming usual mixed rations. Generally, they were present, especially in samples from calf rumens, in sufficiently limited numbers to permit detecting variations in concentrations. Furthermore, they were among the rumen bacteria which can be identified most readily in stained smears in so far as morphology and staining characteristics permit.

In the previous report (4), four of these microorganisms were referred to as being associated with a high proportion of hay ingestion. Large Gram-positive cocci in closely knit pairs were described, and these were referred to as the first hay-group bacteria. The following three bacteria made up the second hay-group and were described as (a) large Gram-positive, thick, fairly square-ended rods, (b) very large Gram-negative, cigar-shaped rods and (c) smaller Gram-negative, short rods in fours and multiples of four in shapes suggestive of window panes. Medium sized, comparatively thin, Gram-positive rods, which sometimes stained in a granular manner, were among the bacteria observed to be associated with the consumption of high proportions of grain.

The bacteria which were observed to be associated with a high proportion of hay ingestion and also the protozoa sometimes failed to become established in young calves. Segregation of the calves from the older stock which apparently cut off their source of inoculum for some of these microorganisms, and the failure of the calves to ingest combinations of feeds suitable for development of the microorganisms were the two reasons involved. Under some conditions, the lack of usual microorganisms in the rumen appeared to be associated with a lowered state of health in these young animals (2, 4, 5). The feeds involved in the above investigations were dry-feed rations consisting of hay and grain. The present report concerns results of a study of the rumen microorganisms in calves on pasture, both with and without hay and grain supplements.

EXPERIMENTAL

Two groups of Holstein and Jersey calves were used. The first consisted of 12 calves which were tethered each day on lawn pasture beginning at 4 days of age. At night, they were kept in the barn in separate groups. They received per day 0.9 lb. of milk for each 10 lb. of body weight at birth. Half of them received

Received for publication August 18, 1949.

rumen inoculations in the manner described previously (4) when they were 5, 10, 15 and 21 days old, using cud materials from cows on pasture. Some of the calves were fed either mixed hay or a 14 per cent protein grain mixture, or both, free choice while they were in the barn at night.

The second group was composed of six calves which were between 39 and 96 days of age, and which averaged 66 days at the time they were given access to pasture during the day beginning on June 7. They had been receiving milk, grain, and mixed hay. These calves received either whole milk or separated milk during the pasture feeding period. They were kept in the barn at night and were turned out each day on a mixed bluegrass and clover pasture in which growth of coarse types of weeds was fairly heavy in some places. Two of these calves received, in addition, 1.5 lb. per day of the grain mixture beginning on July 2. Four had received rumen inoculations during their first 3 wk. of age, and two of these four were given reinoculations with cud materials from cows on pasture. The rumen of the fifth had become partially inoculated in a natural manner, and the sixth was an uninoculated animal which had been raised in comparative segregation.

Rumen samples were collected repeatedly from both groups and examined in the manner described previously (4). Protozoa were examined in the fresh state and Gram-stained smears were relied on for bacterial observations. The presence or absence of the same bacteria as formerly described was determined and the same designations that had been assigned to these have been followed. An attempt was made to grade the samples according to the relative numbers of these particular microorganisms which were observed; this consisted of making rough estimates of the concentration and assigning values ranging between 0 and 4.

RESULTS

Experiment with young calves. The results of the examinations made on rumen samples collected from the 12 younger calves when they were 3 and 6 wk. old are presented in table 1. The treatment as regards inoculation, the type of feed given each calf, and the ratings assigned to the concentrations of some of the microorganisms in the rumen samples are included.

Protozoa were present in the samples from all six of the inoculated calves at 3 wk. of age and were present in great numbers at 6 wk., but were completely absent in the samples obtained from the uninoculated calves. Of the bacteria which had been observed to associate with hay ingestion (4), those in the first hay-flora group were present in samples from all but one of the inoculated calves at 3 wk. of age and in all at 6 wk. They were present in samples from two of the six uninoculated calves at 3 wk. and in those from four of them at 6 wk. of age. Bacteria designated as belonging to the second hay-flora group were present in samples from only one of the six inoculated calves at 3 wk. and in but four at 6 wk. of age. The two calves in whose rumen samples they were absent were receiving grain free choice. These bacteria were never observed in samples from the six uninoculated calves. Within their respective groups, samples from the

TABLE 1

Ratings indicating the relative concentration of certain microorganisms in rumen samples from young calves following access to pasture beginning at 4 days of age

		Rumen microorganisms ratings* at:					
Calf	Feed	3 wk. of age			6 wk. of age		
		Protozoa	Hay-flora		Protozoa	Hay-flora	
			I	II		I	II
Inoculated							
1 H	Pasture alone	3	2	0	3	3	2
2 J	Pasture alone	2	3	1	3	3	2
8 H	Plus hay	2	3	0	3	4	3
Uninoculated							
3 H	Pasture alone	0	0	0	0	1	0
15J	Pasture alone	0	0	0	0	0	0
9 H	Plus hay	0	2	0	0	2	0
Inoculated							
4 J	Plus grain	2	1	0	3	1	0
5 H	Plus grain	1	0	0	3	1	0
11J	Plus hay and grain	3	2	0	3	3	1
Uninoculated							
6 J	Plus grain	0	1	0	0	1	0
14H	Plus grain	0	0	0	0	1	0
12J	Plus hay and grain	0	0	0	0	0	0

* Ratings: 1 = few; 2 = moderate numbers; 3 = many; 4 = masses.

two calves receiving hay in addition to the pasture were rated as having the higher concentrations of the bacteria noted to associate with hay ingestion.

Although not shown in table 1, samples from all 12 calves contained varying numbers of the Gram-positive rods designated in the previous report (4) as associated with grain ingestion. These were especially prevalent in samples from calves consuming a high proportion of grain during the first 4 wk.

The general appearance and growth of all the calves was comparatively good. Their gains in weight are reported in an accompanying paper (3). Four calves suffered from attacks of diarrhea, which were of short duration and not severe, and all recovered without treatment. Two of these calves (numbers 5 and 11) had received rumen inoculations and two (numbers 12 and 15) had not; one received pasture alone (number 15), one pasture plus grain (number 5) and the other two pasture plus both grain and hay (numbers 11 and 12).

Three other uninoculated calves which were born during the same period were kept in the barn continuously, were given milk and fed similar hay and grain free choice starting at 14 days of age. The results of examinations of rumen samples were rather similar to those previously obtained for calves handled in this manner (4). Protozoa were missing from all samples, and bacteria of the hay-flora groups were practically absent from all samples. On the other hand, great numbers of the Gram-positive rods observed to associate with a high proportion of grain ingestion were present in all samples. Of these three barn-fed

calves, two suffered from mild attacks of diarrhea of short duration, which desisted without treatment.

Experiment with older calves. Data collected on rumen samples obtained from the older group of six calves on several representative days are presented in table 2. The ratings assigned on the basis of the relative concentration in the rumen samples of the particular microorganisms which were being observed are given. No marked change in the relative numbers of protozoa were noted throughout the period of observation. The two groups of organisms which have been observed to associate with hay ingestion tended to decrease in the samples during the first few days the calves were on pasture, but soon regained their former status. During this period, small Gram-negative short rods were especially prominent in all the samples. When the calves were first turned out, the pasture was particularly lush. Besides these changes in the microflora, the plasma carotenoids also tended to vary considerably (3), indicating that the character of the pasture was more than likely involved in both variations. The calf having a partial inoculation, which was acquired in a natural manner, developed a rumen flora and fauna which appeared quite comparable to those of the inoculated animals after associating with them for approximately 2 wk. Microorganisms in rumen samples from the uninoculated animal failed to become similar to the others, even though they progressed somewhat in this direction. However, characteristic microorganisms readily were established when the animal received an inoculation with cud materials from an older animal on July 30. A rumen sample obtained on August 23 was rated for protozoa as 3, for hay-flora group I as 3, and for hay-flora group II as 2. It was noted that the same rather large Gram-positive rods frequently seen in other uninoculated calves were present in many of the samples obtained from this calf.

The percentage increases in weight on August 2 over the weights on June 2 were 61.0 and 75.5 per cent for the two inoculated calves which received grain part of the time. For those which did not receive grain, they were 53.5 and 62.6 per cent for the inoculated calves, 58.1 per cent for the naturally inoculated calf and 51.7 per cent for the uninoculated animal. The inoculated calf which gained 53.5 per cent was a twin of the uninoculated calf. The latter calf suffered from recurrent mild diarrhea until after it received the rumen inoculation on July 30. Its tail and hind legs were fouled with feces almost continuously, which was seldom observed in any of the other calves. It also had a noticeably rougher hair coat.

DISCUSSION

The observation that the same rumen microorganisms were established as readily in the rumens of calves eating pasture as when they were consuming dry feeds is in line with the findings of others, including Bortree *et al.* (1), that these feeds promote the development of rather similar rumen flora.

The absence in the uninoculated calves of certain characteristic microorganisms which were present in the rumens of inoculated calves indicates that calves having access to pasture are in no better position than those being raised on dry feeds as regards obtaining these microorganisms from sources other than the

bovine rumen. In the case of the younger calves which were eating lawn pasture, the lack of characteristic rumen microorganisms did not appear to be of much consequence in so far as gain in weight (3) or general health was concerned.

The slowness with which the uninoculated calf in the older group developed rumen flora and fauna similar to the others while on pasture with inoculated calves was rather unexpected. The data collected on this single calf cannot, of course, provide more than a slight indication of what may occur in similar animals under such circumstances. However, it does suggest the possibility that failure of characteristic flora and fauna to become established in the rumens of young animals, which are forced to depend upon pasture utilization to meet their nutrient requirements, may limit their ability to efficiently utilize some types of pasture.

The comparatively normal existence which frequently is possible for calves, even though they lack some of the usual rumen microorganisms, probably is due to the fact that substitute organisms can do a creditable job. However, microorganisms that have developed over a long period of time in the environment of the rumen, would be expected to function most efficiently in this organ.

When the role that segregation can play in the control of the spread of some infectious disease organisms is considered, the effect of such a management procedure on the transfer of rumen microorganisms from animal to animal can be appreciated more readily.

SUMMARY

Rumen inoculations with cud materials from cows on pasture were given six of twelve calves which were fed milk and placed on lawn pasture at 4 days of age. Rumen protozoa and certain bacteria, used as indicators of the presence of varieties characteristically associated with a high proportion of hay ingestion, readily were established in all inoculated calves. The bacteria were established in a relatively less degree in two of the calves which received grain supplement free choice. Protozoa did not develop in the uninoculated calves. Some characteristic bacteria became established in four of the six uninoculated calves by 6 wk. of age, but were limited to one of the observed varieties and were relatively few in number.

Characteristic rumen microorganisms became established only in relatively limited numbers in a milk-fed, uninoculated, 2-month old calf after being in a pasture for 7 wk. with four rumen-inoculated calves of similar age. The marked difference in microorganisms was rectified following rumen inoculation. Prior to inoculation, this calf had recurrent mild diarrhea and a comparatively rough hair coat while on pasture, but its percentage gain in body weight was almost equivalent to an inoculated twin.

Characteristic rumen microorganisms can be established in young calves on pasture when they are inoculated with cud materials from older cattle and when grain is not fed in excessive amounts. Calves possibly may be limited somewhat in their ability to utilize certain pastures, if characteristic rumen microorganisms are lacking.

The authors wish to acknowledge the assistance of John Tate, R. L. Johnson and C. E. Knoop in conducting this investigation.

REFERENCES

- (1) BORTREE, A. L., DUNN, K. M., ELY, B. E. AND HUFFMAN, C. F. Preliminary Report on the Study of Factors Influencing Rumen Microflora. *J. Dairy Sci.*, **29**(8): 542-543. 1946.
- (2) HIBBS, J. W. AND POUNDEN, W. D. The Influence of the Ration and Early Rumen Development on the Changes in Plasma Carotene, Vitamin A, and Ascorbic Acid in Young Dairy Calves. *J. Dairy Sci.*, **31**(12): 1055-1060. 1948.
- (3) HIBBS, J. W. AND POUNDEN, W. D. The Influence of Pasture and Early Rumen Development on the Changes in the Plasma Carotenoids, Vitamin A and Ascorbic Acid and the Liver Storage of Carotenoids and Vitamin A of Young Dairy Calves. *J. Dairy Sci.*, **32**(12): 1016-1024. 1949.
- (4) POUNDEN, W. D. AND HIBBS, J. W. The Influence of the Ration and Rumen Inoculation on the Establishment of Certain Microorganisms in the Rumens of Young Calves. *J. Dairy Sci.*, **31**(12): 1041-1050. 1948.
- (5) POUNDEN, W. D. AND HIBBS, J. W. Rumen Inoculations in Young Calves. *J. Am. Vet. Med. Assoc.*, **114**: 33-35. 1949.

PREPARTUM MILKING. III. THE PLASMA LEVELS OF CAROTENE AND VITAMIN A IN CALVES FROM DAMS MILKED PREPARTUM AND IN CALVES FROM DAMS MILKED POSTPARTUM¹

H. D. EATON, R. E. JOHNSON, A. A. SPIELMAN, L. D. MATTERSON AND L. NEZVESKY
Animal Industries Department, Storrs Agricultural Experiment Station, Storrs, Connecticut

Prepartum milking results in a marked decrease in both the carotene and vitamin A content of colostrum (3). Since colostrum contributes a large proportion of these nutrients to the young calf, it is of value to know what effect prepartum milking of the dam has on these metabolites in the young calf. The purpose of this study was to determine the effect of prepartum milking of the dam on the carotene and vitamin A content of the plasma of the neonatal calf. Secondly, these factors were studied in relation to two dietary regimes.

EXPERIMENTAL

Animals. A detailed description of the treatment, changes in certain blood constituents and composition of the colostrum of the dams of the calves used in this experiment has been reported previously (2, 3). Briefly, the dams were divided into four experimental groupings: 1-A, postpartum milked—basal ration; 1-B, postpartum milked—basal ration + 1 million USP units of vitamin A daily for 30 days prior to the calculated parturition date; 2-A, prepartum milked for 10 days prior to calculated parturition date—basal ration; and 2-B, prepartum milked—basal ration + vitamin A. There were nine calves from dams in group 1-A, ten calves from dams in group 1-B, and 11 calves each from dams in group 2-A and group 2-B.

The calves were not allowed to nurse but were removed immediately after birth to individual pens in a separate portion of the barn. There the Ayrshire and Holstein calves received 6 lb. daily of their dams' colostrum and milk for the first wk., and 7, 8, and 9 lb. of herd milk for the second, third, and fourth wk., respectively. Similar values for the Guernsey and Jersey calves were 5, 6, 7, and 8 lb. All colostrum and milk were fed twice daily in nipple pails. At the beginning of the second wk. of age, each calf had free access to water, commercial dry-calf starter and mixed grass and clover hay. Each calf was weighed at birth and at weekly intervals thereafter to 4 wk. of age. In cases of scours, the milk allowance was reduced to one-half; and in three calves it was necessary to inject intravenously, at the rate of 1 grain per lb. of body weight, a 25 per cent solution of sodium sulfamethazine as a therapeutic agent.

Samples and Analyses. Venous blood samples were drawn at birth and at weekly intervals thereafter to 4 wk. of age. The samples immediately were

Received for publication September 6, 1949.

¹ This work was supported in part by the Big-Y-Foundation, Norwich, Conn. and Chas. M. Cox Co., Boston, Mass.

chilled to 4° C. and centrifuged, and plasma carotene and vitamin A were determined by the method of Kimble (7). Standard statistical procedures (5, 10) were used to test for differences between treatments. In the case of liveweight, the method of Wishart (14) was used, as well as methods outlined in Snedecor (10).

RESULTS

Data on the content of carotene and vitamin A in the blood plasma and liveweight at birth, and at weekly intervals to 4 wk. of age, are given in fig. 1 to 3. Parturition milking resulted in lower levels of both plasma carotene and plasma vitamin A. Secondly, the feeding of supplementary vitamin A to the dam prepartum resulted in higher levels of vitamin A in the plasma and in a depression in the levels of carotene in the plasma.

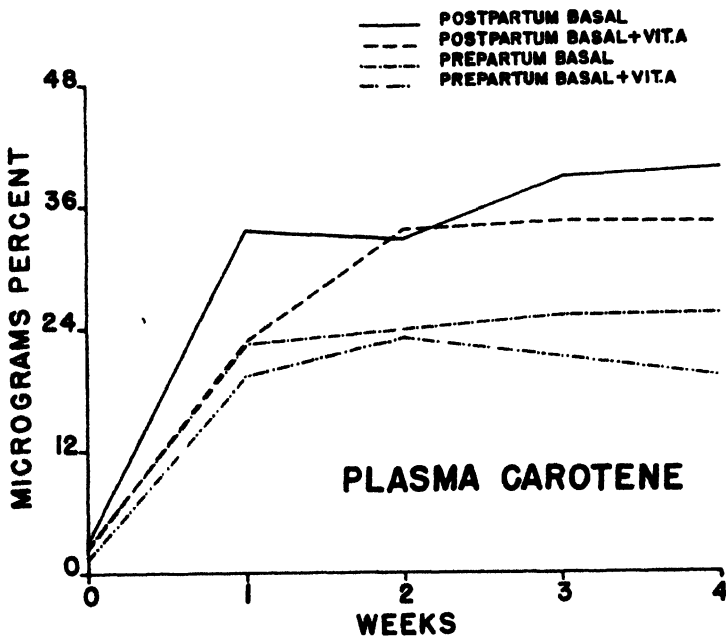


FIG. 1. The effect of prepartum milking of the dam on the carotene content of the plasma of the dairy calf.

Plasma carotene (fig. 1) was affected significantly by treatment. Calves from dams milked prepartum had lower average carotene plasma values ($P < 0.01$) for the entire experimental period, and also lower average values for the period of 1 through 4 wk. of age ($P < 0.05$), than those values for calves from dams milked postpartum only. A similar difference ($P < 0.05$) existed at 1 and at 2 wk. of age. The feeding of supplementary vitamin A prepartum resulted in lower levels of plasma carotene, but these differences were not statistically significant.

Plasma vitamin A levels (fig. 2) were lower ($P<0.05$) from 1 wk. of age through 4 wk. in calves from dams milked prepartum. The feeding of supplementary vitamin A prepartum significantly ($P<0.01$) raised the blood plasma levels of vitamin A for the entire experimental period. Also, the blood plasma levels of vitamin A at birth were higher ($P<0.001$) in those calves from dams fed supplementary vitamin A than in calves from dams fed the basal ration alone.

The liveweight increases (fig. 3) were greater in those calves from dams milked only postpartum and those calves from dams receiving supplementary

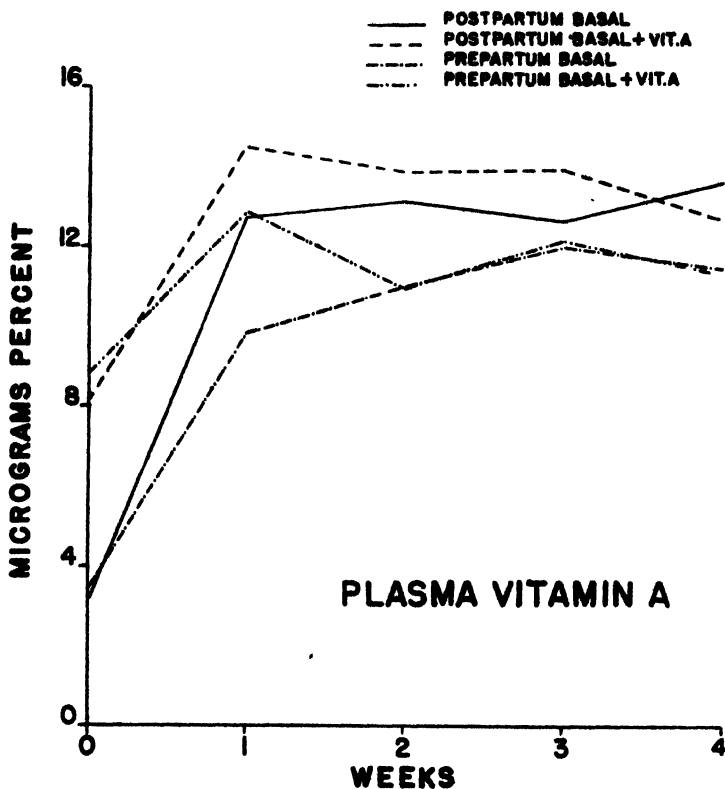


FIG. 2. The effect of prepartum milking of the dam on the vitamin A content of the plasma of the calf.

vitamin A. An analysis of the actual gains during the 28-day experimental period and their linear coefficients, or either of these measures adjusted to the birth weight of the individual calves, failed to reveal statistically significant differences between treatments.

Four calves from dams milked prepartum and fed the basal ration (group 2-A) had scours for a period of 1, 1, 3, and 8 days, respectively. Two of these received intravenous injections of 25 per cent sodium sulfamethazine. In group 2-B, two calves had scours for 1 day each. No scours were observed in calves from

dams milked postpartum and fed the basal ration (group 1-A). One calf in group 1-B had scours for a total of 7 days and received intravenous injections of 25 per cent sodium sulfamethazine. Conversion of the percent days free from scours to an angle corresponding to the percentage, and analysis of variance of the angles showed no statistical differences due to treatment.

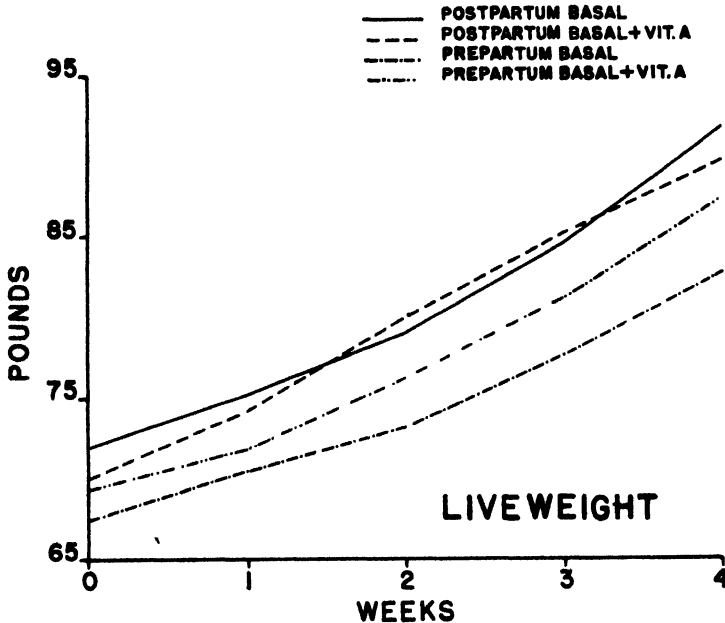


FIG. 3. The effect of prepartum milking of the dam on the liveweight changes of the dairy calf.

DISCUSSION

These data indicate that both management and ration during the prepartum period can influence the blood plasma levels of carotene and vitamin A in the young dairy calf.

Previous workers (6, 8, 13) have reported difficulty in raising calves from cows milked prepartum, and Keyes *et al.* (6) have indicated that oral administration of a carotene preparation would alleviate scours and general inactivity in calves from dams milked prepartum. In this study, the blood plasma levels of vitamin A of the calves from dams milked prepartum and receiving the basal ration alone were, on the average, slightly above 10% per cent, which Boyer *et al.* (1) have indicated as adequate. There appears to be the possibility that a suboptimum intake of vitamin A might exist in calves when their dams receive limited amounts of carotene in their ration and are milked prepartum. However, other factors may influence the nutrition of the calf when its dam is prepartum-milked since not only is there a quantitative change in the constituents of colostrum, but

also a qualitative change as reviewed elsewhere (3). Although the vitamin A content of the colostrum of the dams milked prepartum and fed supplementary vitamin A was significantly greater than that for dams milked postpartum and fed no supplementary vitamin A (3), calves from dams in the former group did not maintain as high plasma vitamin A levels after 2 wk. of age as did calves from dams in the latter group. This suggests that colostrum contains a factor(s), apparently not found in milk, which results in greater efficiency in the utilization of vitamin A. Previous workers (4, 11) have indicated such, but more direct measurements are needed.

The increase in blood plasma levels of vitamin A in those calves from dams fed supplementary vitamin A during the prepartum period confirms work previously reported (12). In addition, the cases of scours, although few and not statistically significant, are in line with the previous report. The depression in carotene, likewise not statistically significant, is of interest; since intrauterine influences of supplementary vitamin A feeding apparently carry over into the neonatal calf under "normal" conditions of feeding and management.

The greater but not statistically significant liveweight gains in calves from dams fed supplementary vitamin A prepartum is of interest. Previous workers (9, 12) have demonstrated significantly greater liveweight gains in neonatal Holstein calves due to prepartum feeding of supplementary vitamin A, and in Holstein heifers fed supplementary vitamin A directly. The lower but not significant weight gains in those calves from dams milked prepartum well might be due in part to suboptimum intakes of vitamin A.

SUMMARY

The effect of prepartum milking of the dam, for 10 days prior to the calculated parturition date, on the plasma carotene and vitamin A levels, liveweight changes, and incidence of scours in 41 young dairy calves has been studied. Secondly, the effect of feeding one million USP units of vitamin A daily for 30 days prepartum was measured.

The data indicate that prepartum milking significantly lowers the level of blood plasma carotene and vitamin A in calves from 1 wk. through 4 wk. of age, as compared to those values for calves from dams milked postpartum only. The feeding of supplementary vitamin A prepartum resulted in significantly greater blood plasma levels of vitamin A for the entire experimental period and lower but not statistically significant blood plasma carotene levels. The differences between treatments, in liveweight and incidence of scours, were not statistically significant.

ACKNOWLEDGMENTS

The authors are most grateful to F. Warren and G. Farrington for the care of the experimental animals and to Misses R. J. Caverno and M. W. Dicks for technical assistance at various times during the course of the experiment. Further acknowledgment is due C. I. Bliss, Storrs Agricultural Experiment Station Biometrician, for suggestions in the statistical analyses of the data.

REFERENCES

- (1) BOYER, P. D., PHILLIPS, P. H., LUNDQUIST, N. S., JENSEN, C. W., AND RUPEL, I. W. Vitamin A and Carotene Requirements for the Maintenance of Adequate Blood Plasma Vitamin A in the Dairy Calf. *J. Dairy Sci.*, **25**: 433-440. 1942.
- (2) EATON, H. D., JOHNSON, R. E., HELMBOLDT, C. F., SPIELMAN, A. A., MATTERSON, L. D., JUNGHER, E. L., KRAMER, J. H., AND SLATE, R. J. Prepartum Milking I. The Effect of Prepartum Milking on Some Blood Constituents of the Cow. *J. Dairy Sci.*, **32**: 870-876. 1949.
- (3) EATON, H. D., JOHNSON, R. E., SPIELMAN, A. A., MATTERSON, L. D., AND NEZVESKY, I. Prepartum Milking II. The Effect of Prepartum Milking on the Carotene and Vitamin A and Proximate Composition of Colostrum. *J. Dairy Sci.*, **32**: (in press). 1949.
- (4) ESH, G. C., SUTTON, T. S., HIBBS, J. W., AND KRAUSS, W. E. The Effect of Soya-Phosphatides on the Absorption and Utilization of Vitamin A in Dairy Animals. *J. Dairy Sci.*, **31**: 461-478. 1948.
- (5) FISHER, R. A., AND YATES, F. Statistical Tables for Biological, Agricultural, and Medical Research. 3rd ed. Hafner Publishing Company, Inc., New York, 1948.
- (6) KEYES, E. A., REID, J. J., BECHDEL, S. I., BORLAND, A. A., BEAM, A. A., BEAM, A. L., AND WILLIAMS, P. S. Prepartum Milking. (An abstract) *J. Dairy Sci.*, **27**: 638-639. 1944.
- (7) KIMBLE, M. S. The Photocolorimetric Determination of Vitamin A and Carotene in Plasma. *J. Lab. Clin. Med.*, **24**: 1055-1065. 1939.
- (8) RAGSDALE, A. C., WEBER, C. W., AND TURNER, C. W. Influence of Milking Pregnant Animals Before Calving on the Physical Condition and Well-Being of the Progeny. *Mo. Agr. Expt. Sta. Bull.* 272. 1929.
- (9) ROSS, R. H., AND KNOTT, C. B. The Effect of Supplemental Vitamin A Upon the Growth, Blood Plasma Carotene, Vitamin A, Inorganic Calcium, and Phosphorus of Holstein Heifers. *J. Dairy Sci.*, **31**: 1062-1067. 1948.
- (10) SNEDECOR, G. W. *Statistical Methods*. 4th ed., Iowa State College Press, Ames. 1946.
- (11) SPIELMAN, A. A., EATON, H. D., LOOSLI, J. K., NORTON, C. L., AND TURK, K. L. (unpublished data) Department of Animal Husbandry, Cornell University. 1947.
- (12) SPIELMAN, A. A., EATON, H. D., LOOSLI, J. K., AND TURK, K. L. The Effect of Prepartum Vitamin A Supplementation on the Health and Performance of the Young Calf. *J. Dairy Sci.*, **32**: 367-374. 1949.
- (13) TURNER, C. W. The Development of the Mammary Gland as Indicated by the Initiation and Increase in the Yield of Secretion. *Mo. Agr. Expt. Sta. Research Bull.* 156. 1931.
- (14) WISHART, J. Statistical Treatment of Animal Experiments. *Supp. J. Royal Statistical Soc.*, **6**: 1-22. 1939.

ASSOCIATION ANNOUNCEMENT

COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Los Angeles, Cal.—Oct. 23, 1949

Teams from 18 State Agricultural Colleges, participated in this, the fifteenth annual Contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

Following is a list of those who won high standings in the Contest:

ALL PRODUCTS

Individuals

1. Herbert Ruggles, Iowa State College
2. Richard Jackson Stanley, Mississippi State College
3. Russell J. Moe, University of Minnesota
4. Donald E. Miller, University of Illinois
5. John R. Tedford, University of Connecticut
6. Gene D. Lower, Ohio State University
7. Lee R. Morgan, Utah State Agricultural College
8. Harold A. Ramsey, Kansas State College
9. Sam Louis Swett, Mississippi State College
10. Robert K. Wight, Iowa State College

Teams

1. Mississippi State College
2. University of Connecticut
3. Iowa State College
4. Kansas State College
5. University of Minnesota
6. University of Massachusetts
- Tie 7. Michigan State College
- Tie 7. State College of Washington
9. Utah State Agricultural College
10. University of Illinois

BUTTER

Individuals

- | | |
|--|-------|
| 1. Russell J. Moe, University of Minnesota | 13.25 |
| 2. Raymond G. Otto, University of Minnesota | 14.67 |
| 3. Herbert Ruggles, Iowa State College | 14.74 |
| 4. Donald E. Miller, University of Illinois | 15.84 |
| 5. Philip J. Blanchard, Jr., University of Massachusetts | 15.92 |
| 6. John R. Tedford, University of Connecticut | 17.00 |
| 7. Duane D. Walter, State College of Washington | 17.50 |
| 8. Warren C. Jones, Texas A & M College | 18.17 |
| 9. William R. Thomas, Oklahoma A & M College | 18.33 |
| 10. Edwin R. Frankel, Michigan State College | 19.00 |

Teams

- | | |
|--------------------------------|-------|
| 1. University of Minnesota | 57.92 |
| 2. University of Massachusetts | 60.93 |

3.	Michigan State College	63.51
4.	University of Connecticut	64.34
5.	State College of Washington	64.51
6.	Mississippi State College	68.10
7.	Kansas State College	68.52
8.	University of Illinois	68.68
9.	Oklahoma A & M College	71.50
10.	Agricultural & Mechanical College of Texas	72.34

CHEESE

Individuals

1.	Sam L. Swett, Mississippi State College	26.08
2.	Richard Jackson Stanley, Mississippi State College	27.59
3.	Dee R. Morgan, Utah State Agricultural College	30.75
4.	John R. Tedford, University of Connecticut	31.02
5.	Dee McDonald Graham, Mississippi State College	31.17
6.	Max R. Hogan, Utah State Agricultural College	31.26
7.	Marvin Eskin, Michigan State College	31.92
8.	James D. Yoder, University of Nebraska	32.51
9.	Alfred Cohn, Michigan State College	32.84
10.	William Edmondson, University of Connecticut	32.93

Teams

1.	Mississippi State College	84.84
2.	Utah State Agricultural College	100.60
3.	Michigan State College	102.00
4.	Iowa State College	104.60
5.	University of Nebraska	105.18
6.	University of Connecticut	106.38
7.	University of Massachusetts	110.43
8.	Kansas State College	110.61
9.	University of Minnesota	111.86
10.	Texas Technological College	112.51

ICE CREAM

Individuals

1.	Roger W. Hunt, University of Connecticut	22.67
2.	Herbert Ruggles, Iowa State College	25.00
	Harold A. Ramsey, Kansas State College	25.84
	Donald E. Miller, University of Illinois	27.34
5.	Richard Jackson Stanley, Mississippi State College	27.67
Tie 6.	Robert K. Wight, Iowa State College	28.00
Tie 6.	Philip J. Blanchard, Jr., University of Massachusetts	28.00
8.	Donald Brighton, University of Idaho	28.50
9.	James A. Brotsos, University of Illinois	29.17
Tie 10.	George L. Weir, Iowa State College	29.50
Tie 10.	Duane D. Walter, State College of Washington	29.50

Teams

1.	Iowa State College	82.50
2.	University of Connecticut	84.84
3.	Mississippi State College	93.18
4.	State College of Washington	93.67

5.	University of Massachusetts	95.00
6.	University of Idaho	95.67
7.	University of Minnesota	95.69
8.	Michigan State College	97.17
9.	Kansas State College	99.85
10.	Ohio State University	100.34

MILK

Individuals

1.	James Howard Sherrod, Kansas State College	12.25
2.	Gene D. Lower, Ohio State University	14.50
3.	Robert K. Wight, Iowa State College	15.67
4.	Russell J. Moe, University of Minnesota	15.92
5.	Richard Jackson Stanley, Mississippi State College	16.42
6.	Dee R. Morgan, Utah State Agricultural College	18.75
7.	James Warren Newell, University of Nebraska	19.25
8.	Harold A. Ramsey, Kansas State College	19.42
9.	William C. Coker, A & M College of Texas	19.75
10.	Donald E. Miller, University of Illinois	19.79

Teams

1.	Kansas State College	52.59
2.	Iowa State College	59.97
3.	Mississippi State College	64.67
4.	State College of Washington	66.42
5.	Agricultural & Mechanical College of Texas	68.42
6.	Utah State Agricultural College	70.00
7.	Ohio State University	70.50
8.	University of Illinois	70.79
9.	University of Connecticut	70.92
10.	University of Minnesota	71.09

MEMBERS AND STUDENT AFFILIATES OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

November 1, 1949

Honorary Members:

Borland, A. A., 310 S. Burrowes St., State College, Pa.
Ellenberger, H. B., Univ. of Vermont, Burlington, Vt.
Frandsen, J. H., Massachusetts State College, Amherst, Mass.
Guthrie, E. S., Cornell Univ., Ithaca, N. Y.
Hayden, C. C., Ohio Agr. Expt. Station, Wooster, O.
Hunziker, O. F., 103 7th Ave., La Grange, Ill.
Mortensen, M., Iowa State College, Ames, Iowa
Reed, O. E., Bureau of Dairy Industry, U. S. D. A., Washington, D. C.

Life Members:

Breed, Robert S., 6 Sunset Drive, Geneva, N. Y.
Harding, H. A., Baileys Harbor, Wis.
Hastings, E. G., Route 3, Box 456, Orlando, Fla.
Larson, C. W., Tudor Plaza Apt., 741 W. Ferry St., Buffalo, N. Y.
Cunningham, O. C., Dairy Dept., College of Agr., State College, N. M.
Rogers, L. A., 3635 S Street N. W., Washington 7, D. C.
Ross, H. E., Cornell Univ., Ithaca, N. Y.
White, George C., Willowbrook Rd., Storrs, Conn.

ALABAMA

Members:

AUTREY, KENNETH M., Dept. of Dairy Husbandry, Alabama Poly. Institute, Auburn
BURKE, A. D., Dept. of Dairy Husbandry, Alabama Poly. Institute, Auburn
CANNON, ROBERT Y., Dept. of Dairy Husbandry, Alabama Poly. Institute, Auburn
EATON, W. H., Dairy Dept., Alabama Poly. Institute, Auburn
FRANKLIN, V. D., 108 Hawthorne Rd., Homewood
HOWLE, HENRY W., Deep South Creamery, Cullman

KIRCHOFF, GEORGE, Bureau of Food & Dairy Inspection, Dept. of Health, Birmingham
PARRISH, J. R., Extension Dairyman, Alabama Poly. Institute, Auburn
ROLLINS, GILBERT, Dairy Dept., Alabama Poly. Institute, Auburn
ROMING, JOHN A., Deep South Creamery, Cullman
TEAGUE, C. E., 1010 Brown Marx Bldg., Birmingham
WIDEMIRE, E. L., Sylacauga

Student Affiliates:

OWEN, FOSTER G., Route 2, Northport

ARIZONA

Members:

BANKS, A. E., Box 156, Glendale
BOWLES, HAROLD O., 2730 Picadilly Ave., Phoenix
CRUMBAKER, JAMES W., P. O. Box 394, Tempe
DAVIS, R. N., College of Agr., Tucson
EWING, JAMES W., JR., Route 5, Box 162, Tucson
HARLAND, F. G., Hopi Lodge, Univ. of Ariz., Tucson
RASKOB, J. J., JR., Route 5, Box 226, Tucson
ROBERTS, LUCIUS E., JR., 2025 Oracle Rd., Tucson

RUST, PHILIP F., Route 2, Box 412, Phoenix
SCARNELLA, E. L., Kraft Foods Co., Phoenix
VAN SANT, W. R., Univ. of Ariz., Agr. Extension Service, Tucson
STULL, JOHN WARREN, Dept. of Dairy Husbandry, Univ. of Ariz., Tucson

Student Affiliates:

BEECROFT, DOUGLAS, 1016 E. 4th St., Tucson
HANNON, LILLIAN L., Box 335, Wickenburg
MCCLELLAND, NORMAN P., 2006 E. Lee St., Tucson
SKOLE, RICHARD, Box 91, E. Stadium Dorm, Tucson

Members:

GARRISON, EARL R., Dept. of Animal Industry, Univ. of Ark., Fayetteville
GIFFORD, WARREN, Dept. of Animal Industry, Univ. of Ark., Fayetteville
HOLLEY, A. LEON, 222 Gray Hall, Univ. of Ark., Fayetteville
MEADOWS, CLIFTON E., Box 693, Fayetteville

HEAD, T. P., 222 Gray Hall, Univ. of Ark., Fayetteville
VOELKER, HOWARD, Dept. of Animal Industry, Univ. of Ark., Fayetteville

Student Affiliates:

LAWSON, HASSEL K., Route 4, Fayetteville
LOWE, THOMAS C., 1131 1st St., Helena

CALIFORNIA

Members:

- ADAMSON, MERRITT H., Adohr Farms, 1801 S. La Cienega Blvd., Los Angeles 35
 AHLSTROM, WALTER, 1965 Euclid Ave., San Marino
 ANDERSON, VIVIAN GRACE, 1317 36th St., Sacramento
 BAIRD, HARRY S., 1220 Dover Rd., Santa Barbara
 BECKLEY, MAURICE S., Rm. 201, P. O. Bldg., San Jose 18
 BESEMER, A. M., 1340 St. Charles St., Alameda
 BEVER, ELDRIDGE, 1403 Country Club Blvd., Stockton
 BROCKMEYER, G. H., 1806 Quito Way, La Canada
 BRUECKNER, HERMAN J., 18 Manor Dr., Piedmont 11
 BRUNNER, THEO, 1901 Las Robles Blvd., N. Sacramento
 BULKLEY, GEORGE S., Carnation Co., Box 2035, Wilshire LaBrea St., Los Angeles
 CHRISTENSEN, SOREN, 2847 Stoddard St., San Bernardino
 CLARY, PAUL D., JR., Route 3, Box 294, Petaluma
 CLEMONS, HARPER B., 5425 Brynhurst Ave., Los Angeles 43
 COLE, H. H., Div. of Animal Husbandry, Univ. of Cal., Davis
 COLE, WILFORD C., Arden Farms, Inc., Research Laboratory, 1900 W. Slauson Ave., Los Angeles
 DETUREK, L. E., 3845 Butler Ave., Fresno
 DEY, MARCUS, Golden State Co., Ltd., 1120 Towne Ave., Los Angeles 21
 DUNCAN, JAMES F., 1562 Waller St., San Francisco 12
 DUNKLEY, W. L., Div. of Dairy Industry, Univ. of Cal., Davis
 ELLISON, HOWARD, 1639 N. Main St., Los Angeles 12
 ENGLE, BRUCE Q., 6418 California St., San Francisco 21
 ERWAY, JOHN, Dairy Industry, Univ. of Cal., Davis
 FASSETT, W. W., 3949 E. Pacific Ave., Sacramento
 FOLEY, DAN, P. O. Box 1391, Vallejo
 FREIDEL, EDWARD H., Arden Farm & Co., Patterson
 FULLER, STEWART A., P. O. Box 685, Arcata
 GARDNER, MORRIS A., 3631 Pomeroy St., Los Angeles 33
 GORDON, G. E., 8 Giannini Hall, Univ. of Cal., Berkeley
 GOULD, JACK S., 3715 Euclid Ave., San Diego
 GREENE, SAM H., 1570 3rd Ave. N., Walnut Creek
 HANSEN, VERNON, Crystal Cream & Butter, Box 1319, Sacramento
 HASLER, GEORGE L., 118 Peralta Way, Fresno 4
 HAUTTER, ALVIN A., 2480 N. Eastern Ave., Los Angeles 32
 HAWKINS, ERVIN E., Good Humor Co. of California, 999 W. Washington Blvd., Los Angeles
 HENDERSON, J. LLOYD, Golden State Co., Ltd., San Francisco 11
 HEYL, RICHARD E., 2511 Webster St., Palo Alto
 HUBBELL, BRUCE, JR., B & 11th St., Davis
 HUESCH, HAROLD, 3615 Jewell, San Diego 9
 JACK, E. L., Div. of Dairy Industry, Univ. of Cal., Davis
 JAMISON, EUGENE O., Route 1, Box 344, Visalia
 JONES, ROBERT E., c/o Pacific Dairy Review, 500 Sansome St., San Francisco
 KELLER, L. G., 360 N. Carmelina Ave., Los Angeles
 KOHLER, ROLAND, 2320 Richelieu Ave., Los Angeles 32
 KREHBIEL, LEONARD F., 3720 Hughes Ave., Los Angeles 34
 LACHMANN, ALFRED, Route 1, Box 100, Davis
 LAMPERT, L. M., P. O. Box 1266, Sacramento
 LEVITAN, MEYER, 1200 Midwickbill Dr., Alhambra
 LOPGREEN, G. P., Animal Husbandry Dept., Univ. of Cal., Davis
 MACKENZIE, F. E., 6130 Avalon Blvd., Los Angeles 3
 MCCORMICK, D. R., Avonset Co., Gustine
 MCFARLAND, G. G., 3126 S. Guirado Ave., Whittier
 MEAD, SYLVESTER W., c/o Agr. Expt. Station, Davis
 MELHUIS, VERN P., 9100 W. 18th St., Los Angeles 35
 MILNER, F. W., 145 S. Spring #900, Los Angeles 12
 MOJONNIER, ROBERT W., 323 E. Foothill Blvd., Altadena
 MORGAN, NORMAN C., Morgan Ice Cream Co., 1822 E. Valley Blvd., Rosemead
 NELSON, VICTOR, Central Research Dept., Food Mach. Corp., San Jose
 NEWTON, ERNEST E., Creameries of America, 325 Roosevelt Bldg., Los Angeles
 PAINTER, W. E., 1949 3rd Ave., Sacramento
 PEEBLES, DAVID D., c/o Western Condensing Co., 411 Battery St., San Francisco
 PHILLIPS, CLEMENT A., Univ. of Cal., Davis
 REGAN, W. M., Agr. Expt. Station, Davis
 ROADHOUSE, C. L., Univ. of Cal., Davis
 ROLEN, WALLACE, Manteca
 RUSSELL, O. M., Box 908, Los Banos
 SARTORI, JAYDEN J., Box 492, Merced
 SEIPP, EDWIN A., JR., Commercial Research, Golden State Co., Ltd., 425 Battery St., San Francisco 11
 SHAMBERGER, DAN, 1161 Irwin, Belmont
 SHARP, PAUL F., College of Agr., Agr. Expt. Station, Univ. of Cal., Berkeley 4

SHELDON, F. M., 1097 S. Marcngo Ave., Pasadena 2
 SHIPSTEAD, HELGE, Div. of Dairy Industry, Univ. of Cal., Davis
 SPERRY, G. D., 3425 Wallace, San Diego 10
 STABILE, JOSEPH N., 406th Medical General Lab., A. P. O. 500, c/o Postmaster, San Francisco
 STACHWICK, ROBERT, 1639 N. Main St., Los Angeles
 STEWART, AUBREY P., JR., Golden State Co., 425 Battery St., San Francisco.
 STOCKLIN, CHALLENGE CREAM & BUTTER ASS'N., 929 E. 2nd St., Los Angeles 12
 TARASSUK, N. P., Dairy Industry Division, Univ. of Cal., Davis
 TRANMAL, HENRY, c/o Arden Farms Co., 1900 W. Slauson Blvd., Los Angeles
 TURNBOW, G. D., 1106 Broadway, Oakland 7
 VON LANGE, OSCAR, Borden's Dairy Delivery Co., Soledad
 YALE, M. W., Arden Farms Co., Patterson
 YOUNG, GEORGE D., JR., 4401 18th St., San Francisco 14

Student Affiliates:

BERG, HERBERT M., 410½ 3rd St., Apt. 2, Marysville
 BERGAN, PAUL A., 2851 E. 5th St., Long Beach 14
 BOYER, JOHN E., 106 Summit Drive, Corte Madera
 BROWNE, ROGER W., 719 Winchester Drive, Burlingame

COHEN, HAROLD, 1319 N. Ridgewood Place, Hollywood 28
 COLLINS, EDWIN B., Div. of Dairy Industry, Univ. of Cal., Davis
 COPPES, JACK, 843 Loring St., San Diego 9
 DODDS, BYRON, 9807 Glasgow Place, Los Angeles 45
 FRAZEUK, DEAN, 3005 Sacramento St., Apt. 2, San Francisco
 HSUEH, TEH YING, South Hall, Univ. of Cal., Davis
 HUTTON, JERRY T., 436 I St., Davis
 JENNINGS, WALTER G., Apt. K 16, Aggie Villa, Davis
 MADDEN, DALE E., 1501 Perris Hill Rd., San Bernardino
 NEDDERMAN, JOHN H., K-10 Aggie Villa, Davis
 NIELSEN, ROBERT L., 4175 Alpha St., San Diego 2
 PURSER, GORDON, 1254 10th Ave., San Francisco 22
 TELXEIRA, JOHN, c/o Coop., Univ. of Cal., Davis
 THILL, GEORGE R., Aggie Villa F-11, Davis
 THOMAS, ROSS G., P. O. Box 151, Ione
 THOMASON, EDMUND, P. O. Box 386, Davis
 TODD, TIM M., 15137 S. Woodruff Ave., Bellflower
 TOWNSEND, E. C., Box 539, Davis
 TUNE, EWING A., JR., Route 2, Box 2800, W. Sacramento
 WAKEFIELD, CHARLES M., c/o Coop., Univ. of Cal., Davis
 WEINBERGER, ISRAEL, c/o Coop., Univ. of Cal., Davis

CANADA

Members:

BAKER, JAMES L., 20 Windley Ave., Toronto 10, Ont.
 BERRY, JOHN C., Dept. of Animal Husbandry, Univ. of B. C., Vancouver, B. C.
 BIRD, W. J., Dairy Supervisor, Nova Scotia Agr. College, Truro, N. S.
 BREMNER, JAMES, Canadian Jersey Cattle Club, 749 Yonge St., Toronto 5, Ont.
 CHAMBERLAYNE, E. C., Dept of Health & Public Welfare, 320 Sherbrook St., Winnipeg, M.
 CRAHTRIE, G. H., Box 372, Abbotsford, B. C.
 DARCH, JOHN KENNETH, c/o S. F. Lawra-son & Co., Ltd., 639 Nelson St., London, Ont.
 GIBSON, A. L., Head of Province Dairy School, Kemptville Agr. School, Kemptville, Ont.
 HANSON, R., 10105 82nd Ave., Edmonton, Alb.
 HAY, J. A., Colony Farm, Essondale, B. C.
 HODGINS, C. L., 100 Adelaide St. W., Toronto, Ont.
 HOOD, E. G., Chief Dairy Research, Central Experiment Farm, Ottawa, Ont.
 IRVINE, O. R., Ontario Agric. College, Guelph, Ont.
 JACKSON, J. R., Simcoe, Ont.

JONES, W. F., The Borden Co., Ltd., 393 Somerset St., Ottawa, Ont.
 KEAY, JOHN, 530 Public Bldg., Calgary, Alb.
 KILLICK, C. H. P., Dairy Branch, Dept. of Agr., Legislative Bldg., Winnipeg, M.
 LICHTER, MAX, Roblin, M.
 MAYBEE, G. R., Fry-Cadbury, Ltd., 2025 Masson St., Montreal 34, Q.
 MERCIER, ERNEST, Quebec Artificial Breeding Center, St. Hyacinthe, Q.
 MORRISON, J. S., The Borden Co., Ltd., Milk Products Division, Tillsonburg, Ont.
 NESS, A. R., MacDonald College P. O. Q.
 OKULITCH, G. J., Fraser Valley Milk Prod. Association, 425 8th Ave., N.W., Vancouver, B. C.
 PEARSON, A. M., 69 Fairview Blvd., Guelph, Ont.
 RATHBY, G., Animal Husbandry Dept., Ontario Agr. College, Guelph, Ont.
 REED, PERCY E., Saskatchewan Dept. of Agr., Legislative Bldg., Regina, Sask.
 SINGLETON, J. F., Assoc. Director, Marketing Service Dairy Prod. Div., Dept. of Agr., Confederation Bldg., Ottawa, Ont.
 STARR, ARTHUR E., Canada Packers, Ltd., Produce Dept., 60 Paton Rd., Toronto, Ont.

STRYNADKA, NICHOLAS JOHN, c/o Dominion Dairy Produce, New Immigration Bldg., Edmonton, Alb.
 THORNTON, H. R., Dept. of Dairy Husbandry, Univ. of Alberta, Edmonton, Alb.
 WASSON, F. C., Dairy Commissioner, Dept. of Agr., Victoria, B. C.

WEBSTER, H. G., 90 Perry St., Woodstock, Ont.
 WOOD, G. ROGER, 2448 Cambie St., Vancouver, B. C.
 YATES, C. MONTAGU, Maxwellton Farm, St. Anne de Bellevue P., Q.

COLORADO

Members:

ALM, VERNE, 632 Martindale Drive, Denver 10
 BEACH, F. LEONARD, 3521 Milwaukee, Denver 5
 GASSNER, FRANK X., Colo. Agr. Expt. Station, Colo. A & M College, Ft. Collins
 MCKELLAR, E. K., Dairy Dept., Colo. A & M College, Ft. Collins
 MILLER, M. M., Univ. of Denver, University Park, Denver 10
 MORTENSEN, O. L., 2145 Market St., Denver 2
 PALMER, W. HORACE, 4550 S. Sherman, Englewood
 POE, CHARLES F., Univ. of Colo., Boulder
 REEVES, ROBERT H., Carlson-Frink Co., Denver
 SANDHOUSE, H. A., Extension Dairyman, Colo. A & M College, Ft. Collins
 STAYER, HARRY B., Dairy Commissioner, 20 State Museum Bldg., Denver 2
 TESELLE, E. A., 2517 9th Avenue Court, Greeley
 WILLIAMS, E. B., Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins

Student Affiliates:

BALLOW, E. E., 625 Stover, Ft. Collins

BROWN, KENNETH D., 82 Mesa St., Veterans' Village, Ft. Collins
 ELMER, ROSS F., Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins
 FOREMAN, JOHN, 412 Garfield, Ft. Collins
 HOWE, R. C., 524 W. Laurel St., Ft. Collins
 HOWELLS, J. J., 2690 S. Lafayette, Denver
 HUMPHREY, SAM, c/o Lucerne Milk Co., 190 W. Nevada St., Denver
 JOBERG, N. A., Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins
 O'LEARY, L. J., 523 Remington, Ft. Collins
 PAUL, ALTON, Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins
 PAUL, NORMAN, Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins
 REY, S. F., 1214 Sylvan Court, Ft. Collins
 SANDERS, ROBERT, 816 Baltimore, Trinidad
 SANDUSKY, CRAIG C., Route 4, Box 310, Greeley
 TETS, OTIS, Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins
 TURNER, LEON, 501 Cowan, Ft. Collins
 WIELAND, S. V., Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins
 WOLFE, ARDEN, Dept. of Animal Husbandry, Colo. A & M College, Ft. Collins

CONNECTICUT

Members:

ANDERSON, E. O., Univ. of Conn., Storrs
 ANDERSON, WILLIAM E., c/o Eastern States Farmers Exchange, Westbrook Farm, R. D. 2, Rockville
 CAVERNO, ROBERTA J., Bldg. E., Univ. of Conn., Storrs
 CHAPMAN, LEROY M., 293 White St., Danbury 7
 CURRUE, GEORGE O., III, R. R. 1, Gerald Park, South Coventry
 DAVIS, MYRON C., R. D. 1, Stafford Springs
 DOWD, LEONARD R., Storrs
 EATON, HAMILTON D., Animal Industry Dept., Univ. of Conn., Storrs
 ELLIOTT, F. I., Dept. of Animal Industry, Univ. of Conn., Storrs
 FREEDMAN, MICHAEL, Box 331, Cheshire
 GOLD, THEO. S., Cream Hill Farm, West Cornwall
 HANSEN, HENRY M., Box U 40, Univ. of Conn., Storrs
 HESSELTINE, W. R., Storrs
 JENNINGS, LEE LAY, 465 S. Main St., West Hartford

JOHNSON, ROBERT E., Univ. of Conn., Storrs
 MACLEOD, HELEN PATRICIA, Univ. of Conn., Storrs
 MANN, ALBERT I., Dairy Dept., Univ. of Conn., Storrs
 MARLAND, RICHARD E., Univ. of Conn., Storrs
 MERRELL, GEORGE G., R. F. D. 1, Plainfield
 NEUMANN, H. D., New Haven Dairy Div., General Ice Cream Corp., New Haven
 SEREMET, JOHN S., Connecticut Milk Producers Assoc., 990 Wethersfield Ave., Hartford 6
 SPIELMAN, ARLESS, Dept. of Dairy Industry, Univ. of Conn., Storrs
 WALKER, WILLIAM R., Dairy Div., Univ. of Conn., Storrs
 WHITE, GEORGE C., Willowbrook Rd., Storrs
 WHITHAM, G. E., R. F. D. 6, Norwich
 WILLMANN, JOSEPH, Derby

Student Affiliates:

MOYLE, WALLACE A., JR., 15 Highland Ave., Darien

CUBA

Members:

COWLEY, PAUL F., Carlos Tercero 1117,
Havana

DELAWARE

Members:

BAKER, THOMAS A., Univ. of Del., Newark

YOUNG, DELMAR J., Dairy Extension Office,
Univ. of Del., Newark

DISTRICT OF COLUMBIA

Members:

BAIN, J. B., 1302 Geranium St. N.W.
BELL, R. W., 4409 Greenwich Parkway
BLETCH, CHESTER T., 1756 K St. N.W.
BURKEY, L. A., Bureau of Dairy Industry,
U.S.D.A.
CASTLE, BENJAMIN F., 1001 15th N.W.
DAVISON, MAURICE V., 2535 Pennsylvania
Ave. N.W.
ELTING, E. C., Office of Expt. Station,
U.S.D.A.
EVERETT, ROBERT, Albee Bldg., 1426 G St.
N.W.
FOHRMAN, M. H., Bureau of Dairy Industry,
U.S.D.A.
FORD, T. FOSTER, Bureau of Dairy Industry,
U.S.D.A.
HIBBEN, ROBERT C., Int. Assn. of Ice Cream
Mfgs., 1105 Barr Bldg.
HOLM, GEORGE E., Bureau of Dairy Indus-
try, U.S.D.A.
HOLMAN, CHARLES W., 1731 Eye St. N.W.
JAMISON, ORVILLE A., 5024 9th St. N.W.,
Apt. 303
KENDRICK, J. F., Bureau of Dairy Industry,
U.S.D.A.
KREY, CHARLES E., Southern Dairies, Inc.,
60 M St. N.E.
LEFEVRE, P. E., 2535 Pennsylvania Ave.
N.W.
MALKEMAN, JAMES P., Bureau of Dairy
Industry, U.S.D.A.

MANOVILL, ROBERT J., 6519 Piney Branch
Rd. N.W.
NYSTROM, A. B., Bureau of Dairy Industry,
U.S.D.A.
PARKER, JOSEPH B., Dairy Husbandman,
Agr. Research Adm., Bureau of Dairy
Ind.
PEARSON, P. B., Div. of Biology & Medicine,
Atomic Energy Commission
REED, O. E., Bureau of Dairy Industry,
U.S.D.A.
SANDERS, GEORGE P., Bureau of Dairy In-
dustry, U.S.D.A.
SWETT, WALTER W., Bureau of Dairy In-
dustry, U.S.D.A.
TITTSER, RALPH P., Bureau of Dairy In-
dustry, U.S.D.A.
TRIMBLE, CHARLES S., 1443 Holly St. N.W.
WEBB, B. H., Bureau of Dairy Industry,
U.S.D.A.
WHITTIER, EARLE O., Bureau of Dairy In-
dustry, U.S.D.A.
WINDHAM, E. S., 4526 13th St. N.W., Apt. 8
WINTERMEYER, W. E., Bureau of Dairy In-
dustry, U.S.D.A.
WISEMAN, HERBERT G., Bureau of Dairy
Industry, U.S.D.A.
WORK, S. H., Office of Expt. Station,
U.S.D.A.
WRIGHT, PHILIP A., Bureau of Dairy In-
dustry, U.S.D.A.

ENGLAND

Members:

ALTWEGG, C. ALBERT, General Milk Prod-

ucts, Ltd., 116-126 Cannon St., London,
E.C. 4

FLORIDA

Members:

ARNOLD, P. T., Dept. of Animal Husbandry,
Univ. of Fla., Gainesville
ARRINGTON, LEWIS B., Dairy Products Lab.,
Univ. of Fla., Gainesville
BASSETT, CUREY J., Bassett Dairies, Inc.,
Tallahassee
BECKER, R. B., Animal Industry Dept.,
Univ. of Fla. Agr. Expt. Station, Gaines-
ville
BOWEN, J. O., 62 N.E. 27th St., Miami
DRESSSEL, R. L., Dressel's Dairy Farm, P. O.
Box 1656, Riverside Station, Miami 35
DUPUIS, JOHN G., JR., P. O. Box W, Little
River Station, Miami 38
FOGG, E. C., III, 101 Alton Rd., Miami
Beach 39
FOOTS, EVERETT L., Dairy Products Lab.,
Fla. Agr. Expt. Station, Gainesville

HAMMER, B. W., 369 W. Arlington Ave.,
Sarasota
HASTINGS, E. G., Route 3, Box 456, Orlando
KRIENKE, WALTER A., Univ. of Fla., Gaines-
ville
MARSHALL, SIDNEY P., Dept. of Animal In-
dustry, Univ. of Fla., Gainesville
MULL, LEON E., Dairy Products Lab., Univ.
of Fla., Gainesville
NEAL, WAYNE M., c/o Jackson Grain Co.,
P. O. Box 1290, Tampa 1
PARSON, C. H., 3613 W. Platt St., Tampa
REAVES, C. W., Extension Dairyman, Univ.
of Fla., Gainesville
SCRIBNER, L. A., D.V.M., City Hall, Orlando
SHEALY, A. L., Animal Industry Dept.,
Univ. of Fla., Gainesville
STOY, CHARLES O., Dade County Health
Unit, 800 Court House, Miami

TRIPSON, JOHN ROBERT, Vero Beach Dairies,
Vero Beach
TWORGER, GEORGE, Box Q, Little River Sta-
tion, Miami
ULVIN, GUS B., 1733 Sefir Circle West, Jack-
sonville
WILLIAMS, N. KENNETH, P. O. Box 142,
Fort Pierce
WRENSHALL, C. L., Foremost Dairies, Inc.,
53 W. Ashley St., Jacksonville

Student Affiliates:

BALDWIN, L. R., 5212 10th Ave. N., St.
Petersburg
BAXTER, LOREN H., Chi Phi House, Gaines-
ville
BEARMAN, JULIUS E., 1231 W. University
Ave., Gainesville
CAMMACK, ELBERT, 103 N. Hillside, Orlando
CLEMONS, JAMES A., Pi Kappa Phi House,
1469 W. University, Gainesville

DUDLEY, JAMES R., Flavet 111, Apt. 212D,
Gainesville
EDWARDS, OTHO JR., Box 646, Quincy
HENDRIE, JAMES H., 415 S. 9th St., Gaines-
ville
LANEY, WILLIAM A., 5506 Seminole Ave.,
Tampa 4
LEWIS, JOHN, 900 N.W. 180th St., Miami
LEWIS, RICHARD, 3248 Green St., Jackson-
ville
MINEAR, LAIRD, Kappa Sigma House,
Gainesville
MINEAU, JUDSON, Box 217, Jupiter
POTTER, WOODROW P., Broward Grain & Sup-
ply Co., Ft. Lauderdale
SANCHEZ, ALDON B., Star Route, Old Town
SCHEE, L. B., P. O. Box 2812, University
Station, Gainesville
SCHNEIDER, RUDY, JR., Box 545, Eustis
WEAVER, CURTIS A., Box 313, Boynton
Beach
WILLIAMS, CHARLES R., 217 T Flavet 111,
Gainesville

GEORGIA

Members:

BONAR, HENRY, Moultrie
BRYAN, WILLIAM G., c/o Rosemary Cream-
ery, 62 Baltimore Place N.W., Atlanta
CALLAWAY, FULLER E. JR., Hills & Dales,
Lagrange
CLARK, GEORGE J., Columbus Field Training
Station, Muscogee County Health Dept.,
Columbus
COARSEY, RALPH W., Riegeldale Farm, Trion
DAVIS, LUKE, P. O. Box 953, Atlanta 1
DEHART, G. H., Dept. of Health, 815 Hem-
lock St., Macon
GAY, JAMES D., 661 Whitehall St., Atlanta
GRIFFON, GEORGE R., Route 2, Madison
HARRIS, J. J., Pelham
HARRIS, L. A., Assistant County Agent,
Wilkes County, Washington
HAWKINS, JOE K., 314 Main St., Lagrange
HENDERSON, H. B., Univ. of Ga., Athens
MASSEY, Z. A., Georgia Expt. Station, Ex-
periment
MECH, A. W., Kraft Foods Co., 890 Me-
morial Dr. S.E., Atlanta
MORRISON, SPENCER H., Dairy Dept., College
of Agr., Univ. of Ga., Athens
SANCKEN, GEORGE A., Box 710, Augusta

SHEURING, JOHN J., Dept. of Dairy Hus-
bandry, Univ. of Ga., Athens
SOUTHWELL, B. L., Georgia Coastal Plain
Expt. Station, Tifton
STRATTON, JAY W., Washington
WARD, C. A., Rucliff Farm, R.F.D. 3, Athens
WEISSMAN, HARRY, U. S. Penitentiary,
Atlanta
WELLS, BERT H., The Coca Cola Co., P. O.
Box 1734, Atlanta
WILSON, WILLIAM L., Wilson Dairy Prod-
ucts, 657 E. Lake Dr., Decatur

Student Affiliates:

ARREDALE, JOHN V., JR., Dairy Dept.,
Univ. of Ga., Athens
BENNETT, F. W., Dairy Dept., Univ. of Ga.,
Athens
CONNER, JOHN SIDNEY, R.F.D. 1, Monroe
FOLDS, GEORGE R., Waleska
GOBER, WILLIAM, 304 Cherokee St., Marietta
MAHAFFEY, J. C., Youngcane
MARET, KARL, Dudley Hall, Univ. of Ga.,
Athens
MCGOWAN, JOHN, Box 121, Washington
MORGAN, ROBERT, 1394 S. Milledge Ave.,
Athens
MYERS, FRED, P. O. Box 76, Marietta
PERRY, ROY, Route 2, Colbert

GREECE

Student Affiliates:

STROGYLI, PARAZKEVI, 68 Spetson St.,
Athens

Members:

HENKE, L. A., Agr. Expt. Station, Univ. of
Hawaii, Honolulu

HAWAII

Members:

ANDERSON, G. C., State House, Boise
BOYD, JAMES CREIL, Dept. of Dairy Hus-
bandry, Univ. of Ida., Moscow

IDAHO

BUCK, CHARLES, 119 S. 1st W., Rexburg
FOURT, D. L., Dept. of Dairy Husbandry,
Univ. of Ida., Moscow

HANSEN, HENRY C., Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 KERRY, DARRELL C., Caldwell Branch Expt. Station, Route 4, Caldwell
 MANWARING, D. H., Banquet Better Foods, Nelson-Ricks Creamery Co., Rexburg
 MCGLOSSON, ELMER D., Apt. 1-e, North Main Village, Moscow
 ROSS, RICHARD H., Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 SLATER, I. W., 1208 Owyhee St., Boise
 THEOPHILUS, P. R., College of Agr., Moscow
 TRASK, E. S., Upper Snake River Valley Dairymen's Assn., Inc., Box 501, Idaho Falls

Student Affiliates:

ALLDAFFER, ROBERT, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 BEAL, ERNEST, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 BISHOP, ROBERT, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 BUSH, MILAN, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 COPENHAVER, HOWARD V., Dept. of Dairy Husbandry, Univ. of Ida., Moscow

DALKE, ORLANDO, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 DEMOTT, BOBBY, 716 Lynn, Moscow
 GARRETT, ERNEST A., Route 2 North, Pocatello
 JAUSSE, FERD. G., Paris
 KILSGAARD, CARL, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 MILLER, EARL, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 MORGAN, DEE, R.F.D., Ovid
 MYASKI, TOMMY, Route 1, Sugar City
 NESBITT, SHERMAN, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 PLATO, NICK, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 SENFTEN, EUGENE R., 1400½ Washington Ave., Boise
 SHEPHERD, WILLIAM G., Box 772, Montpelier
 SUTTER, JOHN, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 THACKER, DAVID, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 TRAUTMAN, JACK, Dept. of Dairy Husbandry, Univ. of Ida., Moscow
 WILLIAMS, BILL, Dept. of Dairy Husbandry, Univ. of Ida., Moscow

ILLINOIS

Members:

ACKERMAN, H. A., Food Materials Corp., 2521 W. 48th St., Chicago 32
 ALEXANDER, M. H., 120 Old Agr. Bldg., Univ. of Ill., Urbana
 ANDERSON, RALPH J., Siskrafft Co., 205 N. Wacker Dr., Chicago
 BARNES, R. E., 327 W. Prairie, Marengo
 BARON, MORRIS, 6606 S. Richmond St., Chicago Lawn, Chicago
 BOARD, F. S., 162 E. Ohio, Chicago
 BOBER, L. E., Babson Bros. Co., 2843 W. 19th St., Chicago
 BOYLE, REID G., 223 W. Park St., Edwardsville
 BRUGGS, S. E., 20 N. Wacker Dr., Chicago 6
 BROCK, JOHN H., Box 128, Dundee
 BROWN, J. C., American Butter Institute, Room 1404, 110 N. Franklin St., Chicago 6
 BROWN, W. CARSON, 100 Elmhurst Rd., Box 274, Prospect Heights
 BRYANT, H. WAYNE, 504 N. Willis St., Stockton
 BURROWS, O. K., Cherry-Burrell Corp., 427 Randolph St., Chicago 6
 CARTER, E. B., Meadow-Gold Dairies, 304 S. Main St., Decatur
 CHRISTIANSEN, C. VALDIMAR, 140 W. Ontario St., Chicago
 CORBETT, W. J., 1724 Ridge Ave., Rockford
 DARGER, HARRY C., 2022 Lincoln St., Evanston
 DENNERLEIN, ARNO A., The Quaker Oats Co., 141 W. Jackson Blvd., Chicago 4
 DIAMOND, WILLIAM T., American Feed Mfg. Assoc., 53 W. Jackson Blvd., Chicago

EDMAN, GEORGE J., 703 E. Center St., Leroy
 EISENSTEIN, NORMAN, 1263 Pratt Blvd., Chicago
 ELDER, R. E., A & P Tea Co., 211 W. Wacker Dr., Chicago 6
 ELLICKSON, BRUCE E., 1713 N. State St., Westville
 ELMSLIE, W. P., Moorman Mfg. Co., Quincy
 EPSTEIN, ALBERT K., 5 S. Wabash, Chicago
 FAIRBANKS, B. W., American Dry Milk Institute, 221 N. LaSalle St., Chicago
 FAIRCHILD, F. C., Prairie Farms Creameries, 43 E. Ohio St., Chicago 11
 FEDDERSON, R. E., 3931 S. Leavitt St., McKinley Park Station, Chicago
 FELDMAN, SHELDEN, 2811 W. Division, Chicago 22
 FIFER, RUSSELL, American Butter Institute, 110 N. Franklin St., Chicago 6
 FLAKE, J. C., 1321 Washington, Evanston
 FOX, WILLIAM K., 918 Hickory St., Waukegan
 FRITZ, JAMES C., 22 Monroe St., Elgin
 FREYMAN, LEO R., 1016 W. Vine, Champaign
 GAINES, W. L., 120 Davenport Hall, Univ. of Ill., Urbana
 GARDNER, KARL, Dept. of Dairy Husbandry, Univ. of Ill., Urbana
 GAYMONT, STEPHEN, 320 N. LaSalle St., Chicago
 GIBSON, GILBERT G., 10541 Drew St., Chicago 43
 GOODMAN, MARK C., JR., Goodman American Corp., Federal at 45th St., Chicago 9
 GRAHAM, T. W., Allied Mills, Inc., 210 Suffolk Bldg., Decatur
 GRANDISON, EDWARD G., Swift & Co., U. S. Yards, Chicago

- GREENSPAN, JOSEPH, Consolidated Products Co., 119 N. Washington Ave., Danville
 GUNDERSON, N. O., 8th Floor, City Hall Bldg., Rockford
 HAMAN, ROBERT W., The Wander Co., Villa Park
 HAMILTON, GEORGE B., 20 N. Wacker Drive, Chicago 6
 HANSEN, ELMER N., Curtiss Candy Co., Cary
 HARPER, CLAUDE, JR., Beatrice Foods Co., 1526 S. State St., Chicago 5
 HARRIMAN, L. A., Armour Research Lab., Union Stocks Yards, Chicago
 HARSHBARGER, K. E., 120 Davenport Hall, Univ. of Ill., Urbana
 HEDRICK, T. I., 626 S. Maple Ave., Oak Park
 HEMB, D. M., 3659 Harrison St., Chicago
 HENRY, J. W., 221 N. La Salle St., Chicago
 HERRED, ERNEST, Dept. of Dairy Husbandry, Univ. of Ill., Urbana
 HETRICK, J. H., Dean Milk Co., Rockford
 HOEGER, VERNON C., Box 792, Warrenville
 HORNE, L. W., 500 Peshtigo St., Kraft Foods Co., Chicago
 HORRALL, E. E., 6759 S. Oglesby Ave., Chicago 49
 HOYT, RICHARD M., 807 S. Main St., Normal
 HUFFER, ENOS G., 1328 Noble Ave., Springfield
 HULL, MAURICE E., 230 S. Catherine Ave., LaGrange
 HUNNICUTT, W. D., National Dairy Products Co., Inc., 75 E. Wacker Drive, Chicago 1
 HUNZIKER, O. F., 103 Seventh Ave., La Grange
 HUSSONG, R. V., Kraft Foods Co., 923 Waukegan Road, Glenview
 INGLE, JAMES D., 9825 S. Peoria St., Chicago
 JACOBSEN, D. H., Cherry-Burrell Corp., 427 W. Randolph St., Chicago
 JENSEN, OTTO G., 112 N. Home Ave., Park Ridge
 KARNOPP, KARL W., 216 N. Spring St. Waukegan
 KEEFER, KYLE W., c/o Sprague Dairy Co., Lockport
 KEITEL, KLAUS, Millstadt
 KELLER, FLOYD M., 309 W. Jackson Blvd., Chicago
 KENDALL, K. A., Dairy Husbandry Dept., Univ. of Ill., Urbana
 KENT, O. B., The Quaker Oats Co., Liberty Villa Farm, Libertyville
 KLEIN, RALPH A., 1526 S. State St., Chicago
 KUEBLER, JUSTA, 706 Maple Ave., Downers Grove
 KUNKEL, REINOLD, Kraft Foods Co., Research Lab., Glenview
 KWASIGIOCH, CARL, Ass't. County Agent, Farm Bureau, McHenry Co., Woodstock
 LERNER, L., Phil Kalech Research Lab., 200 E. Illinois St., Chicago 11
 LONG, HENRY F., Sugar Creek Creamery Co., Danville
 LONGSDORF, JOHN H., 2817 Auburn St., Rockford
 LOOS, HENRY, Kraft Foods Co., Glenview
 LOUDER, EARL A., Pet Milk Co., Greenville
 LOY, WILLIAM C., Wilson & Co., 41st St. & S. Ashland Ave., Chicago 9
 MAACK, ARTHUR C., 10905 S. California Ave., Chicago 43
 MAINA, MYETLE, Kraft Foods Co., 923 Waukegan Rd., Glenview
 MARTENS, CARL N., 907 S. Forrest Ave., Carbondale
 MARTIN, ETHEL A., c/o National Dairy Council, 111 N. Canal St., Chicago
 MCAULIFFE, HERBERT, Bowman Dairy Co., 140 W. Ontario St., Chicago
 MCKINNEY, WILLIAM B., Beatrice Foods Co., 120 S. LaSalle St., Chicago 3
 MERHOFF, JOHN H., c/o Borden Co., Marengo
 NAFIS, LOUIS F., c/o Georgian Hotel, Evanston
 NEVENS, W. B., College of Agr., Urbana
 NORTH, G. C., Beatrice Creamery Co., 1526 S. State St., Chicago
 O'MALLEY, C. M., American Dry Milk Institute, Inc., 221 N. LaSalle St., Chicago
 ORMISTON, E. E., Dept. of Dairy Prod., Univ. of Ill., Urbana
 OSRI, STANLEY M., 4100 N. Kenneth Ave., Chicago
 OVERMAN, O. R., Dairy Dept., Univ. of Ill., Urbana
 PARFITT, E. H., Evap. Milk Assoc., 307 N. Michigan Ave., Chicago 1
 PARKER, MILTON E., Room 512A, 308 W. Washington St., Chicago 6
 PASHKOW, A. D., 415 W. Scott St., Chicago 10
 PEDRICK, R. F., Dean Milk Co., 1126 Kilburn Ave., Rockford
 POPE, DONALD B., 703 N. Evans, Bloomington
 PROHASKA, J. R., 2555 W. Diversey Ave., Chicago 47
 PRUCHA, MARTIN J., 702 Nevada, Urbana
 PUTNAM, GEORGE W., 1042 Pontiac Rd., Wilmette
 PYENSON, HARRY, Dairy Manufactures Bldg., Univ. of Ill., Urbana
 RAUSCH, KARL H., 1021 S. 3rd St., Galena
 REEDER, GEORGE W., 5446 S. Dorchester, Chicago
 REICHAERT, E. L., Rm. 1312, 110 N. Franklin St., Chicago 6
 REINBOLD, GEORGE W., JR., 1005 W. University, Urbana
 REMALEY, ROBERT J., 320 Spring St., Park Ridge
 RHODE, C. S., Dept. of Dairy Prod., Univ. of Ill., Urbana
 RICE, FRANK, Evaporated Milk Assoc., 307 N. Michigan Ave., Chicago
 RICHARDS, OWEN M., American Dairy Ass'n., 20 N. Wacker Dr., Chicago
 RISHOR, A. H., Cherry-Burrell Corp., 427 W. Randolph St., Chicago

ROBICHAUX, R. P., 4333 N. Winchester, Chicago 13
 ROUNDY, ZOLA DOYLE, 17 McIntosh Ave., Clarendon Hills
 RUEHE, PROF. H. A., 908 S. Lincoln Ave., Urbana
 SALISBURY, GLENN, Dept. of Dairy Husbandry, Univ. of Ill., Urbana
 SAMPSON, JESSE, 509 W. Charles St., Champaign
 SCHRENK, O. J., Bowman Dairy, 140 W. Ontario, Chicago
 SCHROEDER, HERMAN C., 7938 Crandon Ave., Chicago
 SCOTT, E. C., Ashton
 SHADWICK, G. W., JR., Beatrice Creamery Co., 1526 S. State St., Chicago 5
 SIERRE, LEWIS, The Diversy Corp., 53 W. Jackson Blvd., Chicago
 SIEHRS, A. E., Krim Ko Corp., Chicago
 SIMMONS, NICHOLAS L., Stock-Gro, Inc., 333 N. Michigan Ave., Chicago
 SMITH, F. R., Pet Milk Co., Greenville
 SMITH, HIRAM P., 1257 Granville Ave., Apt. 3W, Chicago 40
 SMITH, WARREN, R., Dairy Dept., Univ. of Ill., Urbana
 SNOW, C. H., P. O. Box 77, Bloomington
 SNYDER, VICTOR P., 16231 Emerald Ave., Harvey
 SOMMER, W. A., Borden's Soy Processing Co., P. O. Box 508, Kankakee
 SPANNUTH, H. T., 5458 Dorchester, Chicago 15
 STERNITZ, V. C., Chicago Dairy & Food Lab., 6930 N. Clark St., Chicago
 STEVENSEN, FRENTON G., Breese
 STINE, J. BRYAN, Kraft Cheese Co., Research Lab., 923 Waukegan Rd., Glenview
 STOKES, LOUIS, 5551 S. Kimbark Ave., Chicago 37
 STOLPER, ERNST G., 18 Bailey Rd., Park Forest, Chicago Hts.
 STORRS, ARNOLD B., Box 13, Palos Heights
 SYNOLD, R. E., Soyfood & Oil Corp., 422 S. Front St., E. St. Louis
 TRACY, P. H., Dept. of Food Technology, Univ. of Ill., Urbana
 TUCKEY, S. L., 101 Dairy Manufactures Bldg., Univ. of Ill., Urbana
 TURNER, J. RAY, Preferred Brands Co., 461 W. Erie St., Chicago 10
 VANDEMARK, N. L., Dept. of Dairy Prod., Univ. of Ill., Urbana
 VORHES, CARL A., Q. M. F. & C. I., 1845 W. Pershing Rd., Chicago 9
 WAINESS, HAROLD, U. S. Public Health Service, 69 W. Washington, Chicago
 WARNER, E. A., Sunbeam Corp., 5600 Roosevelt Rd., Chicago 50
 WATTS, MEREDITH W., Allied Mills, Inc., Box 459, Libertyville

WEINER, L. H., Borden Co., Chicago Milk Division, 3638 Broadway, Chicago 13
 WEINREICH, CHARLES F., c/o Cherry-Burrell Co., 427 W. Randolph St., Chicago
 WHITNEY, ROBERT MCL., Dept. of Food Technology, Univ. of Ill., Urbana
 WHITNEY, W. O., 1243 W. Washington Blvd., Chicago
 WILSON, H. KENNETH, Wilson Ice Cream Co., 107 E. Elm St., Urbana
 WILSON, H. L., Kraft Cheese Co., 500 Peshigo Court, Chicago 90
 WRIGHT, J. HAROLD, Research Lab., Pet Milk Co., Greenville
 WOLFELEY, DONALD C., White Hall
 YAPP, W. W., 608 W. Green St., Urbana
 YOUNG, PAUL A., 75 E. Wacker Drive, Chicago 1
 ZIMMERMAN, HARLIE F., 218 Barker Ave., Peoria 5

Student Affiliates:

ALBRECHT, JOHN R., 1108 W. Springfield, Urbana
 BREEDLOVE, ROYCE, Dairy Dept., Univ. of Ill., Urbana
 COE, ROBERT, Box 54, Shabbona
 DAILY, HOWARD, R.F.D. 2, Peoria 5
 DIDIER, AL J., 1525 Maryland Drive, Urbana
 GROSCHKE, CHARLES A., 109 Phillippa Ave., Waukegan
 HEIRES, PAUL, Frazer Apts., Chester
 HUGHES, CHARLES A., 1109 W. Stoughton, Urbana
 HUNDING, CARL W., 208 W. Columbia, Champaign
 KELLY, MAX B., 312 S. State., Champaign
 KIBLER, WILLIAM T., 401 Barr Ave., Jerseyville
 KREUZKAMP, LINCOLN, 512 E. Healey, Champaign
 MCBRIDE, CLIFFORD, 456 W. Judd St., Woodstock
 MCCABE, PAUL J., Ludlow
 MODENHAUER, RAYMOND, Box 144, Monmouth
 MOORE, DONALD, Dept. of Food Technology, Univ. of Ill., Urbana
 RUESTMANN, D. G., 705 W. Elm St., Apt. 8, Urbana
 SANG, DONALD G., 1025 N. Lathrop Ave., River Forest
 SCHMIDT, A. R., 5545 Rosemont, E. St. Louis
 SISLER, WILLIAM, Ohio
 SMITH, MAX, 501 E. Springfield, Champaign
 SOUTHWELL, LEONARD, 403 2nd St., Pana
 VARNNEY, HOWARD, 1117 W. Illinois Ave., Urbana
 WESTLAKE, ORVILLE O., Producer's Dairy, S. College St., Danville
 ZIVNET, S. E., 128 Locust St., Minonk

INDIA

Student Affiliates:

KALAPA, C. D., Dairy Development Dept., Fort Mercara P. O., Coorg

KONAR, M. R., Khoshbagan Burdwan, W. Bengal
 MATHUR, P. E., Animal Husbandry Dept., Bikaner

INDIANA

Members:

BABEL, FRED J., Dairy Husbandry Dept.,
Purdue Univ., Lafayette
BARNETT, BEN F., Silver Lake
BARNETT, J. A., R. R. 2, Box 2, Boswell
BRYAN, KENNETH V., Smith Hall, Purdue
Univ., Lafayette
CHUMLEA, L. W., Associated Laboratories,
810 N. Meridian St., Lebanon
COHENOUR, FRANCIS D., c/o Armour & Co.,
Rochester
COLE, GLENN H., c/o Swift & Co., Marion
DE LACROIX, FRANK E., 1718 Northwestern
Ave., Lafayette
EICHENBERGER, WILLIAM R., 666 Hendricks
St., Berne
GALEMA, M. L., R. D. #1, Lafayette
GANNON, E. A., Purdue Univ., West Lafay-
ette
GRAVES, R. D., P.O. Box 392, Valparaiso
GREGORY, H. W., Chief of Dairy Husbandry,
Purdue Univ., Agr. Expt. Station, Lafay-
ette
HARVEY, JOHN E., JR., 716 E. Woodside St.,
South Bend
HIGLEY, PHILIP I., 1939 Northwood Drive,
Indianapolis 20
HOLLENDER, H. A., Dept. of Dairy Hus-
bandry, Purdue Univ., Lafayette
IDDINGS, HARRY G., Route 1, Box 185, Gary
JOHNSON, K. R., Smith Hall, Purdue Univ.,
Lafayette
LARSON, R. A., Indiana Dairy Prod. Assoc.,
623-25 Board of Trade Bldg., Indian-
apolis 4
LUNDQUIST, NORMAN S., Dept. of Dairy
Husbandry, Purdue Univ., West Lafay-
ette
MCPEAK, T. L., 360 Penn Ave., Ft. Wayne

MILLER, NORMAN J., Mead Johnson & Co.,
Evansville
MOODY, E. GRANT, Dept. of Dairy Husban-
dry, Smith Hall, Purdue Univ., Lafayette
MOSLEY, W. K., 3862 E. Washington St.,
Indianapolis 1
OVERMAN, ORTON, 357 N. Walnut St., Union
City
PARSONS, DONALD W., 256 W. 3rd, Peru
PRICKETT, P. S., Mead Johnson & Co.,
Evansville
ROBERTS, R. E., 918 Robinson Ave., W.
Lafayette
SCHARF, IRVIN C., Purity Maid Prod. Co.,
New Albany
SIEGRIST, URBAN J., St. Joseph's College,
Collegeville
STARR, GEORGE W., Red 73 Creamery, Inc.,
Union City
STOUT, JOSEPH L., 415 North St., Lafayette
SUHRE, F. H., Columbus
TEMBLE, J. M., Lebanon
TRUE, N. F., Mead Johnson & Co., Evans-
ville 21
WEISKETTEL, WALTER A., Goshen Milk Cond.
Co., Goshen
WERLING, WILLIAM H., 861 Lincoln Ave.,
Evansville 13
WILBUR, J. M., Smith Hall, Purdue Univ.,
Lafayette
WILLIAMS, G. A., Purdue University, W.
Lafayette
ZELL, FRED, Carmel

Student Affiliates:

BROWN, GORDON, 408 Center St., Valparaiso
DIETRICK, LOUIS L., Box 813, Portland
HERREM, ANDREW T., Romney
VANDERBEEK, JAMES L., 113½ West 8th St.,
Rochester

IOWA

Members:

ARNOLD, FLOYD, Extension Service, Iowa
State College, 202 Morrill Hall, Ames
BAKER, MERLE P., Dept. of Dairy Industry,
Iowa State College, Ames
BIRD, EMERSON W., Dept. of Dairy Indus-
try, Iowa State College, Ames
BONEWITZ, PAUL W., Bonewitz Chemicals
Inc., P.O. Box 560, Burlington
BURWELL, F. A., Rath Packing Co., Water-
loo
CANNON, C. Y., Animal Husbandry Dept.,
Iowa State College, Ames
CAULFIELD, W. J., Dept. of Dairy Industry,
Iowa State College, Ames
EDMONDSON, JOE E., Dept. of Dairy Indus-
try, Iowa State College, Ames
FAHL, JOSEPH R., 1417 East Lombard St.,
Davenport
FINCHAM, ROBERT G., 222 South Hazel,
Ames
GOSS, E. F., Dept. of Dairy Industry, Iowa
State College, Ames
HANSON, F. E., 1321 Ave. H, Hawarden

HUGHES, CHARLES E., Successful Farming,
Des Moines 3
IVERSON, C. A., Dept. of Dairy Industry,
Iowa State College, Ames
JACKSON, LYLE W., 923 16th St. N. E.,
Cedar Rapids
JACOBSEN, N. L., Dept. of Animal Hus-
bandry, Iowa State College, Ames
KRISTOFFERSON, THORVALD, Dairy Industry
Dept., Iowa State College, Ames
KUBICEK, MILTON D., 1145 First St. S. W.,
Mason City
LUSH, JAY L., Agriculture Hall, Iowa State
College, Ames
MORTENSEN, MARTIN, Iowa State College,
Ames
NELSON, F. E., Dept. of Dairy Industry,
Iowa State College, Ames
NIELSEN, VERNER H., Dept. of Dairy Indus-
try, Iowa State College, Ames
OWEN, THOMAS S., 209 S. 12th St., Center-
ville
PARMELEE, C. E., Dairy Industry Dept.,
Iowa State College, Ames

PEEBLES, H. E., Bacteriology Lab., Iowa State Dept. of Agr., State House, Des Moines
 PETERS, ISAAC, Dairy Industry Bldg., Iowa State College, Ames
 PIEIE, J. W., Eastern Artificial Breeding Assoc., Cedar Rapids
 PORTER, ARTHUR R., 110 Curtiss Hall, Iowa State College, Ames
 RAPS, GREG, 3108 Ellis, Ames
 RATHORE, A., Animal Husbandry Dept., Iowa State College, Ames
 ROSENBERGER, W. S., Dairy Industry Dept., Iowa State College, Ames
 RUDNICK, A. W., 214 S. Russell Ave., Ames
 SANDERS, L. R., Hutchinson Ice Cream Co., Cedar Rapids
 THOMAS, B. H., 306 Chemistry Bldg., Iowa State College, Ames
 VOELKER, DONALD E., 202 Morrill Hall, Iowa State College, Ames
 WARE, ROBERT, Carroll Creamery Co., Carroll
 WEAVER, RUSSELL C., 310 S. Tennessee, Mason City
 WEGERMANN, EDWIN H., 864 18th St. S. E., Cedar Rapids
 WILKOWSKA, HOWARD, Dairy Industry Dept., Iowa State College, Ames

Student Affiliates:

ACKER, ROBERT M., 310 Ivanhoe Rd., Waterloo
 ANDRAKE, JOHN, 217 Ash, Ames
 BAKER, R. J., Dairy Industry Dept., Iowa State College, Ames
 BASSETT, PAUL, Box 175, Station A, Ames
 BELL, KENNETH, 307 15th St. N. W., Mason City
 BRAYMAN, DONALD, 124 N. Hyland Ave., Ames
 CALVERT, PHILLIP, R. F. D. 4, Jefferson
 CHRISTENSEN, JAMES V., 2120 Lincoln Way, Ames
 COFFIN, DALE, Rutland
 COYLE, CHARLES, 466 Pammel Ct., Ames
 DAVIES, IDRIS, Alumni Hall, Iowa State College, Ames
 DAVIES, JAMES, 1329 Kirkwood Ave., Iowa City
 DUDANI, A. T., Dairy Industry Dept., Iowa State College, Ames
 GEORGE, KENNARD, 307 W. 4th St., Boone
 GERNAND, MAX, Volga City
 GERRY, KEITH, 17th & Early, Sac City
 GEEVAIS, HUGH B., 2110 Lincoln Way, Ames
 GIDEONSEN, RAY, 1933 S. Bluff, Clinton
 GOODMAN, RICHARD, McCallsburg
 HAGEN, VERNON, Joice

HARVEY, WALTER R., Animal Br. Section, Iowa State College, Ames
 HAUPT, ERNEST, 1818 Boone St., Boone
 HENRYSON, ROY, 228 Welch, Ames
 HERBICK, JOHN, 1219 Lincoln Way, Ames
 HILDRETH, DONALD G., 744 Pammel Ct., Ames
 HUMPHREY, SAM, 145 Pammel Ct., Ames
 JOHANSON, RICHARD E., 304 Welch Ave., Ames
 JONES, GAIL M., 310 Michigan Ave., Storm Lake
 JONES, MILO, Corydon
 KEEN, PAUL, 1118 Jones St., Bettendorf
 KELLOGG, WALLACE, 713 Pammel Ct., Ames
 KOELE, EARL, 84 Pammel Ct., Ames
 LARSEN, CYRIL, Box 27, Akron
 LEGATES, J. E., 670 Pammel Ct., Ames
 LOVLIN, CARL, 303 Welch Ave., Ames
 LUND, WAYNE E., 211 N. Hazel, Ames
 MAGILL, ROBERT, Hughes Hall, Ames
 MAGRAB, M., Station A, Box R, Ames
 MCBRIDE, CLIFFORD, 131 Campus Ave., Ames
 MCGILLIARD, LON D., Animal Breeding Sub-section, Dept. of Animal Husbandry, Iowa State College, Ames
 MEYERS, WILLIAM, 62 May St., Manning
 MOELLER, RICHARD, 107 S. Riverside Dr., Ames
 MONAWECK, JOHN, 2112 Lincoln Way, Ames
 MORRIS, WALTER, Strawberry Point
 MORRISON, HENRY F., 1137 Northwestern, Ames
 NASHIF, SELIM A., Box 330, Friley Hall, Ames
 NELSON, WALTER, Randall
 NORRIS, HOOD, 304 Welch Ave., Ames
 OLSON, ROBERT A., Model Dairy, Waukon
 PATGH, ROBERT J., 325 Welch, Ames
 PHELPS, THOMAS, 733 Pammel Ct., Ames
 PLATT, THOMAS B., Osborne Cottage, Iowa State College, Ames
 RHODES, SAM, 311 Ash Ave., Ames
 SKOGMAN, DEANE, Farmersburg
 STODDARD, GEORGE E., Dept. of Dairy Husbandry, Iowa State College, Ames
 THOMAS, ROBERT H., Dairy Industry Dept., Iowa State College, Ames
 VITTENGLE, JOHN G., 2424 Lincoln Way, Ames
 WAGGONER, BURDETTE, 630 7th St. S. E., Cedar Rapids
 WEIR, GEORGE, 403 W. Lincoln Way, Apt. 3, Ames
 WEYANT, ROBERT E., Strawberry Point
 WILHELM, DENNIS, Route 1, Renwick
 YANG, S. P., Dept. of Animal Husbandry, Iowa State College, Ames
 ZEUTHEN, NIELS, International House, Iowa State College, Ames
 ZIMMERMAN, HOWARD, Dairy Industry Dept., Iowa State College, Ames

IRELAND

Members:

GRIMES, MICHAEL, Dairy Bacteriology Dept., University College, Cork

ISRAEL

Student Affiliates:

POLISHUK, MENASHE, 137 Rotshild St.,
Petah-Tiqua

KANSAS

Members:

ATKESON, F. W., Dept. of Dairy Husbandry, Kan. State College, Manhattan
BECK, GLENN H., Dept. of Dairy Husbandry, Kan. State College, Manhattan
BRIGHT, HOWARD, 5351 Ash St., Mission
CHILSON, WILLIAM H., Dept. of Dairy Husbandry, Kan. State College, Manhattan
CLAYDON, T. J., Dairy Dept., Kan. State College, Manhattan
DAY, CLARENCE A., 520 Ash St., Ottawa
ECKLES, WILLIAM CLARENCE, 5706 Ash Dr., Mission
ELDRIDGE, FRANKLIN, Dept. of Dairy Husbandry, Kan. State College, Manhattan
FOREMAN, CHARLES F., 825 Fremont St., Manhattan
FOUNTAIN, F. C., Dept. of Dairy Husbandry, Kan. State College, Manhattan
GEORGE, B. J., Kansas City Power & Light Co., 1830 Baltimore Ave., Kansas City 10
HOSTETLER, P. H., Harper
ISSEN, HEMAN L., Dept. of Animal Husbandry, Kan. State College, Manhattan
KU, BENEDICT H. Y., Dept. of Dairy Husbandry, Kan. State College, Manhattan

LINN, J. W., Extension Division, Kan. State College, Manhattan
MARTIN, WILLARD H., Kan. State College, Manhattan
PARRISH, DONALD B., Dept. of Chemistry, Kan. State College, Manhattan
UNDERBERGER, GRAVERS K. L., Dept. of Physiology, Kan. State College, Manhattan
WHITNAH, CARRELL H., Dept. of Chemistry, Kan. State College, Manhattan

Student Affiliates:

AINSLIE, HARRY, Apt. 28D, Elliott Courts, Manhattan
BARTLEY, ERLE, Dairy Husbandry Dept., Kan. State College, Manhattan
GIGSTAD, ALFRED, Dairy Barn, Kan. State College, Manhattan
QUIMBY, KENNETH, 2100 Madison, Great Bend
RAMSEY, HAROLD, 1409 Fairchild, Manhattan
SHERROD, HOWARD J., 1648 Leavenworth, Manhattan
STOKES, WILLIAM BRICE, 209 W. 23rd, Apt. 8, Wichita
ZECK, MLADDEN, D., 316 4th Ave., Leavenworth

KENTUCKY

Members:

BARKMAN, J. O., Dairy Dept., Univ. of Ky., Lexington
BARKMAN, ROBERT L., Prospect
BLUE, JAMES L., JR., Cudahy Packing Co., Harrodsburg
CARPENTER, MARSHALL C., Route 6, Louisville
CLIFTON, CARL M., Dairy Dept., 730 Rose St. Lexington 29
DEUTSCH, ANN, Route 4, Box 773, Louisville 13
DOZIER, EMMITT, JR., 306 Douglas Ave., Versailles
ELLIOTT, RALPH F., Dairy Section, Univ. of Ky., Lexington
FOSTER, JAMES D., 730 Rose St., Lexington
FOSTER, JOHN W., Dairy Dept., Univ. of Ky., Lexington
FREEMAN, THEO R., Dairy Section, Univ. of Ky., Lexington
HIXSON, E. L., Pet Milk Co., Mayfield
MCCULLOUGH, MARSHALL E., Box 1123 College Station, Berea
MENNEN, K. E., Armour Creameries, Louisville 8
MORRISON, H. B., Dairy Dept., Univ. of Ky., Lexington
SEATH, DWIGHT M., 106 Dairy Bldg., Univ. of Ky., Lexington
SUMME, JOHN J., 224 E. 20th St., Covington

Student Affiliates:

BURNETTE, CECIL, Route 1, Fulton
BUSH, LINVILLE, R. D. 3, Winchester
CONLEY, CECIL, University Apts., 333 South Lime, Lexington
DUNNING, MOSE W., JR., 317 Grosvner Ave., Lexington
DUVALL, CHARLES ALLEN, JR., Smithfield
EARLY, WALTER B., JR., Box 123, Williamsburg
FORTNER, HENRY L., 600 3rd St., Fulton
GRAHAM, JOHN D., Box 546, Hopkinsville
JOHNSON, JAMES T., Route 1, Water Valley
LASSITER, CHARLES, 544 B Hilltop Ave., Cooperstown, Univ. of Ky., Lexington
LEMASTER, JAMES L., 527 Brewer Drive, Cooperstown, Univ. of Ky., Lexington
LINTON, FRANK B., 303 E. Maxwell St., Lexington
MCNEIL, WILLIAM SAMUEL, JR., Box 1253, College P. O., Berea
MULLINS, DEWEY A., 741 Oldham Ave., Lexington
PRICE, H. V., 100 Wabash Place, Apt. 1, Lynn Acres Apt., Louisville 8
RAV, THEODORE S., Box 5634, Univ. of Ky., Lexington
SAALWAECHTER, PAUL, 708 Meredith, Cooperstown, Univ. of Ky., Lexington
SAMPLES, ROBERT N., Route 1, Fancy Farm
SCHOOLCRAFT, GEORGE W., Cain Stone
STAHL, PERRY, Pikeville College, Pikeville

LOUISIANA

Members:

ANDERSON, H. W., Extension Dairyman,
Univ. Station, Baton Rouge
BRANTON, CECIL, L. S. U. Artificial Breed-
ing Center, Dairy Dept., Univ. Station,
Box 8907, Baton Rouge 3
BROWN, CHARLES A., 841 Exposition Blvd.,
New Orleans 15
CALLOWAY, R. C., Univ. Station, Baton
Rouge
CATHCART, S. L., Iberia Expt. Farm, Jeaner-
ette
DAVIS, C. C., 2618 Calhoun St., New Orleans
18
FREY, JENNINGS B., JR., Dairy Dept., La.
State Univ., Baton Rouge
GANTT, PAUL A., Trinity Plantation, Rosc-
dale
GELPI, A. J., JR., Assoc. Prof. of Dairying,
La. State Univ., Baton Rouge
GIBBONS, J. T., P.O. Box 284, New Orleans
HAQ, MD OBAYEDUL, Box No. 8463, L. S. U.,
Baton Rouge 3
MILLER, G. D., Louisiana State Univ., P.O.
Box 7443, Baton Rouge
NEASHAM, E. W., La. State Univ., Baton
Rouge
PATRICK, T. E., Dairy Dept., La. State
Univ., Baton Rouge
RIVERS, PHILIP, Southwestern La. Institute,
Lafayette
ROUGEON, C. L., Dairy Dept., Southwestern
La. Institute, Lafayette
RUSOFF, LOUIS L., Dairy Research, La. State
Univ., Baton Rouge
SMYTH, EUGENE R., 4920 Moore Drive, New
Orleans 19

WARNER, ROBERT A., Route 3, Box 133,
Monroe

Student Affiliates:

ALFORD, JOHN A., Dept. of Bacteriology,
La. State Univ., Baton Rouge
BARKER, HAL B., Box 507, La. Polytechnic
Institute, Ruston
BATEMAN, DALLAS L., Box 8381, La. State
Univ., Baton Rouge
CADWALLADER, J. M., JR., Box 1188, Baton
Rouge
D'ARENSBOURG, GERALD F., Box 6592, La.
State Univ., Baton Rouge 3
ESTESS, D. L., Box 739, Mansfield
EVERAGE, OTIS R., Box 6883, La. State
Univ., Baton Rouge
HODGKINS, E. E., Box 7330 A, La. State
Univ., Baton Rouge
HOUEY, MILES, Box 566, Amite
JAMES, CHARLES B., 2533 Jessamine St.,
Baton Rouge
KUHNELL, J. S., 7428 Freret St., New Or-
leans
MCDANIELS, THOMAS M., Spring Creek
MEADE, E. L., 525 Government St., Baton
Rouge
NOLAN, DONALD J., Box 6406, La. State
Univ., Baton Rouge
SCOTT, G. W., Iberia Livestock Expt. Sta-
tion, Jeanerette
SIMON, DENIS E., JR., Box 6741, La. State
Univ., Baton Rouge 3
WALKER, W. D., JR., Box 8733, La. State
Univ., Baton Rouge
WEST, ERIC E., Lillie
WREN, MARCUS D., Jr., Route 5, Minden

MAINE

Members:

DICKEY, HOWARD, Univ. of Maine, Orono

DORSEY, L. M., Dairy Division, Univ. of
Maine, Orono
ROGERS, L. A., Patten

MARYLAND

Members:

ARBUCKLE, W. S., Dairy Dept., Univ. of
Md., College Park
BARBCK, C. J., 3705 Chevy Chase Lake Dr.,
Apt. 3, Chevy Chase 15
CAIRNS, G. M., Dairy Dept., Univ. of Md.,
College Park
CARY, C. A., 4605 Queensbury Rd., Riverdale
CASKEY, C. D., JR., Southern States Coop.,
2101 E. Fort Ave., Baltimore 30
COLLIER, CHARLES W., Indian Springs Farm,
Darlington
CONVERSE, H. T., Beltsville
ELLMORE, FRANKLIN, Dept. of Dairy Hus-
bandry, Univ. of Md., College Park
ELY, RAY E., Bureau of Dairy Industry,
Beltsville
ENGLAND, C. W., 4408 Underwood St.,
Hyattsville
GIFFORD, JOHN, Box 889, Silver Spring
GLICK, D. P., Box 661, Frederick

HALL, J. OAKLEY, Dairy Dept., Univ. of Md.,
College Park
HALLER, HARRISON S., 28 Everett St.,
Kensington
HETZIOLOS, B. C., Dept. of Dairy Hus-
bandry, Univ. of Md., College Park
HILDER, ROBERT A., Bureau of Dairy Indus-
try, Agr. Research Center, Beltsville
HODGSON, R. E., 7006 Wake Forest Drive,
College Park
JACOBSON, W. C., Bureau of Dairy Industry,
U.S.D.A., Beltsville
KANE, EDWARD A., U.S.D.A., Beltsville
LANG, JULIUS F., Kennersley Farms, Church
Hill
LESCURE, J. M., Fairfield Western Maryland
Dairy, Linden Ave. & Dolphin St., Balti-
more 3
LOCHRY, H. R., Bureau of Dairy Industry,
Beltsville Agr. Research Center, Beltsville
MARTY, IVAN H., Room 902, Municipal
Bldg., Baltimore 3

MATTHEWS, CHARLES A., U. S. Bureau of Dairy Industry Station, National Agr. Research Center, Beltsville
 McDONALD, CHARLES S., 5614 Hamilton Manor Dr., Apt. 2, Hyattsville
 McELWEE, 201 Reynolds Ave., Hagerstown
 MECHAM, C. MARION, 2503 Taylor Ave., Baltimore 14
 METCALF, KENNETH L., Box 459, Frederick
 MOORE, L. A., Bureau of Dairy Industry, Beltsville
 PORTERFIELD, RALPH A., Univ. of Md., College Park
 POTTER, FRANK E., 806 Easley St., Silver Spring
 ROBERTS, HENRY E., Mt. Aarat Farms, Port Deposit
 SAARINEN, PELLERVO, Univ. of Md., College Park
 SCHABINGER, J. R., Ass't. County Agent, Westminster
 SENGEL, MARVIN E., Dairy Dept., Univ. of Md., College Park
 SHAW, JOSEPH C., Dairy Dept., Univ. of Md., College Park
 SHEPHERD, J. B., 9528 49th Ave., Berwyn
 SIEGEL, MAURICE, 1403 Eutaw Place, Baltimore 17
 SIEGMUND, H. B., Hendlar Creamery Co., 1100 E. Baltimore St., Baltimore 2
 STAGG, A. M., 16 Willis St., Westminster
 SYKES, JOSEPH F., U.S.D.A., Bureau of Dairy Industry, Beltsville
 THOMAS, J. WILLIAM, Bureau of Dairy Industry, Beltsville

TREBLER, H. A., 14 E. Chase St., Baltimore 2
 WARREN, F. G., Dairy Husbandry Dept., Univ. of Md., College Park
 WEATHERBY, EDWIN J., 8501 Greenwood Ave., Takoma Park
 WILSON, LOGAN T., Sherwood Feed Mills, Inc., 500 President St., Baltimore

Student Affiliates:

CARUTHERS, BRUCE E., 3608 Longfellow St., Wyattsville
 CHUNG, ANTHONY CHI WU, 7509 Princeton Ave., College Park
 COLEMAN, WILLIAM R., 4302 Knox Rd., College Park
 DRAWBRIDGE, ROBERT FRANCIS, 3308 40th Place, Colmar Manor
 GOETZ, PAUL C., Box 2044, Univ. of Md., College Park
 GREENBERG, MORTON, 213 W. Madison St., Baltimore 1
 HOOKER, ROBERT K., Box 4631, Univ. of Md., College Park
 POLITE, JOSEPH G., Box 2614, Univ. of Md., College Park
 RICHTER, LAWRENCE, 8209 Wilson Ave., Baltimore
 SCHEUTUMPF, ROBERT J., VE 10-B, College Park
 SPENCER, CHARLES, 3043 St. Paul St., Baltimore 18
 SPRING, ARTHUR H., 7107 Chatham Rd., Chevy Chase 15
 WACHTER, EUGENE T., Route 3, Frederick

MASSACHUSETTS

Members:

ABRAHAMSON, CARL E., 45 Queen St., Franklin
 ARCHIBALD, JOHN G., Dept. of Animal Husbandry, Mass. State College, Amherst
 BIRDSEYE, CLARENCE, Eastern Point Blvd., Gloucester
 BURKE, EDMUND W., Lyndonville Creamery Ass'n., 86 Elm St., Watertown
 CAMPBELL, P. A., Eastern States Farmers Exchange, West Springfield
 FAY, A. C., H. P. Hood & Sons, 500 Rutherford Ave., Boston
 FINNEGAN, E. J., Dairy Dept., Univ. of Mass., Amherst
 FOLEY, RICHARD C., Stockbridge Hall, Univ. of Mass., Amherst
 FRANDSEN, J. H., Mass. State College, Amherst
 FREEMAN, STANLEY L., 419 Spring St., West Bridgewater
 GAUNT, STANLEY N., Stockbridge Hall, Mass. State College, Amherst
 GUIMOND, ARTHUR C., 359 Robeson St., Fall River
 HANKINSON, D. J., Dept. of Dairy Industry, Univ. of Mass., Amherst
 HAWKINS, V. M., JR., 53 Pleasant St., Sharon

HOLMES, ARTHUR D., Univ. of Mass., Amherst
 HOWARD, HARLAN A., 750 N. Pleasant St., Amherst
 KEITH, ROBERT H., 75 Indian Hill Rd., Worcester
 KELLER, D. S., 61 Ellington St., Longmeadow
 LINDQUIST, H. G., Dairy Industry Dept., Univ. of Mass., Amherst
 LYTGOE, HERMANN C., 36 Fair Oaks Ave., Newtonville 60
 MARCUS, THEODORE, 577 Blue Hill Ave., Roxbury 21
 MUELLER, W. S., Dept. of Dairy Industry, Mass. State College, Amherst
 NELSON, D. H., Dairy Industry Dept., Mass. State College, Amherst
 RICE, V. A., Mass. State College, Amherst
 SELBY, H. W., United Farms Corp., Creamery Ass'n., Charlestown
 SHRAEDER, J. H., Eastern Nazarene College, Wollaston
 SIBLEY, JOHN R., Spencer
 SMITH, ALBERT W., United Dairy System, Springfield
 SMITH, CHESTER G., Box 141, Hadley
 STEVENS, KENNETH M., 121-123 Randolph St., N. Abington

STITTS, T. G., H. P. Hood & Sons, 500
Rutherford Ave., Boston 29
TOOLEY, HARRY C., Health Dept., Pittsfield
UTLEY, SAMUEL T., E. Washington Rd.,
Hinsdale
WALTS, CHARLES C., H. P. Hood & Sons, Re-
search Dept., 56 Roland St., Boston 29
WARD, RUEL E., Eastern States Farmers
Exchange, West Springfield
WATSON, JAMES G., New England Home-
stead, Springfield
WHITWORTH, JOHN, 40 Garland St., Melrose
76
WILDE, H. G., Highlawn Farm, Lenox
WILLMANN, ALFRED, 165 Clifton St., Bel-
mont
WISE, ROBERT, National Ice Cream Co., Inc.,
166 London St., East Boston 28

Student Affiliates:

BASSETT, EMMETT W., 925 N. Pleasant St.,
N. Amherst
BEMIS, ERNEST W., 86 W. Chestnut St.,
Brockton 69
BLANCHARD, PHILIP J., JR., 50 Hollywood
St., Worcester 3
BOYLE, RICHARD S., 30 Maple Terrace, W.
Springfield
BRUNELL, SYDNEY, 389 N. Pleasant St.,
Amherst
CASH, CLARENCE D., 39 Conway St., Green-
field

MEXICO

Members:

CARRANCO, EDUARDO GARCIA, Calle Alamo
40, Mexico D. F.
LYNCH, P. CAMPOS, Niza 15, Mexico City

MICHIGAN

Members:

ALGER, HARRY B., 19051 Dolores Ave., Birm-
ingham 6
BAILEY, HERBERT, The Whittin, Burns Drive,
Detroit 14
BALTZER, A. C., Extension Dairyman, Dairy
Dept., Mich. State College, E. Lansing
BECHTEL, H. ERNEST, Box 68, North End
Station, Detroit 2
BERNHART, F. W., Wyeth, Inc., Mason
BRUNNER, ROBERT, Dairy Dept., Mich. State
College, E. Lansing
BRYAN, C. S., Surgery & Medicine Dept.,
Mich. State College, E. Lansing
BRYANT, C. B. A., Box 455, Montgomery
BURLINGAME, M. M., 15261 Promenade Ave.,
Detroit 24
BURNS, CLIFTON W., Dairy Dept., Mich.
State College, E. Lansing
CHRISTIANSEN, JAMES B., Box 68, North
End Station, Larro Research Farm, De-
troit 2
COLE, CLARENCE, 4151 McKay Rd., Romeo
COPE, GEORGE, 2111 Riverside Dr., N. W.,
Grand Rapids

CHIN, RICHARD G. L., Food Technology
Dept., Univ. of Mass., Amherst
DEARY, JOSEPH, Box 274, Webster
GAFFNEY, MICHAEL C., 24 Cottage Park,
Reading
JOHNSON, HARRY, Ridge Rd., Rutland
KAYE, ARTHUR E., 35 Buchholz St., Spring-
field
LANGEVIN, HERMAN E., 45 Old Sturbridge
Rd., Southbridge
LIBBEY, LEONARD, 60 Townsend Rd., Bel-
mont
McCARTHY, JEREMIAH A., 159 Calumet St.,
Roxbury
PELUSO, OTIS H., 20 W. Baltimore St., Apt.
1, Lynn
PERRY, ROLLIN W., 74 Rand St., Revere
RONEY, DAVID P., JR., 13 Edmonds St.,
Bromerville
SALVIE, RAYMOND A., 64 School St., Seekonk
SMITH, CLARENCE B., 375 N. Pleasant St.,
Amherst
STEWART, FRANK, 375 N. Pleasant St., Am-
herst
SULLIVAN, JOHN F., 32 Clarendon St., North
Andover
THAYER, PAUL E., JR., 8 Strathmore Rd.,
Worcester
WRABEL, MICHAEL G., 61 Myrtle Ave., Web-
ster
YALOFF, ABRAHAM S., 50 Hamilton Ave.,
Lynn

PRICHARD, ARTHUR, Cremeria Americana 4a
Martires de la Conquista 92 y 94, Tacu-
baya

DANDO, ROBERT E., Box 54, Stephenson
DEW, PAUL R., 114 Buffalo St., Bad Axe
DUNCAN, CLIFFORD W., Dept. of Agr. Chem-
istry, Mich. State College, E. Lansing
DUNN, J. A., Diamond Crystal Salt Co., St.
Clair
DUNN, KENNETH M., Dairy Dept., Mich.
State College, E. Lansing
EWBANK, F. C., Mich. Milk Producers
Ass'n., Imlay City
FABIAN, F. W., Div. of Vet. Science, Mich.
State College, E. Lansing
FARRALL, A. W., Dept. of Agr. Engineering,
Mich. State College, E. Lansing
GIRRBACH, GEORGE, Soo Creamery, Sault St.
Marie
HAYS, J. G., 605 Butterfield Drive, E.
Lansing
HORWOOD, RUSSELL E., Upper Peninsula
Expt. Station, Chatham
HUFFMAN, CARL F., Dairy Dept., Mich.
State College, E. Lansing
JENSEN, J. M., Dairy Dept., Mich. State
College, E. Lansing
JOHNSON, LAWRENCE A., Dairy Dept., Mich.
State College, E. Lansing

JONES, V. R., Wyandotte Chemicals Corp., Wyandotte
 LEWIS, ROBERT C., Dairy Dept., Mich. State College, E. Lansing
 LUCAS, PAUL S., Mich. State College, Dairy Dept., E. Lansing
 LUTZ, WILLIAM B., Upper Peninsula Expt. Station, Chatham
 MACLEOD, JOSEPH W., Northland Dairy Division, General Foods Corp., Ewart
 MCMAHON, HARRY, 8047 Hamilton Ave., Detroit 2
 McMILLAN, DONALD Y., 301 2nd St., E., Flint 3
 MEISER, JOSEPH C., JR., Dairy Dept., Mich. State College, E. Lansing
 METES, JOSEPH, Dept. of Physiology & Pharmacy, Mich. State College, E. Lansing
 MINOR, L. H., 3138 Birchwood, Trenton
 MORLEY, LEWIS W., Mich. Milk Prod. Ass'n., 406 Stephenson Bldg., Detroit
 MURRAY, D. L., Dairy Husbandry Dept., Mich. State College, E. Lansing
 NELSON, RONALD H., Dept. of Animal Husbandry, Mich. State College, E. Lansing
 NOBLITT, C. O., Box 55, College Station, Berrien Springs
 PARSONS, GEORGE E., 202 Dairy Bldg., Mich. State College, E. Lansing
 PETRIE, ERNEST B., Lockshore Farm, Hickory Corners
 RALSTON, NOEL, Dairy Dept., Mich. State College, E. Lansing
 REINEKE, E. P., Dept. of Physiology & Pharmacy, Mich. State College, E. Lansing
 RICHARDS, W. F., Richards Dairy, 205 Brush St., St. Johns
 ROEHM, L. S., Room 255, 47 Bldg., Dow Chemical Co., Midland
 ROGERS, C. A., 8731 Witt St., Detroit
 SALSBURY, R. L., 211 S. Sycamore, Lansing 15
 SEIDEL, C. J., 1838 McKinley Ave., Bay City
 SEIDEL, MARTIN, 403 E. John St., Bay City
 SMITH, WAYNE E., 701 Michigan Ave., Sturgis
 SNYDER, W. W., Dairy Dept., Mich. State College, E. Lansing
 STEPHENSON, ROBERT, 994 61st St., S. E., Grand Rapids 8
 STOUT, ROBERT E., 430 Cedar St., Sault Ste. Marie
 STRAIT, HOWARD, 501 Walker St., Sturgis
 TRAEVER, LESTER, 330 N. Sears St., Reed City
 TROUT, G. MALCOLM, Dairy Dept., Mich. State College, E. Lansing
 WARD, GEORGE M., Dairy Dept., Mich. State College, E. Lansing
 WEAVER, EARL, Dairy Dept., Mich. State College, E. Lansing
 WEBER, DALE K., Box 2007, Mich. Ave. Station, Lansing 11

Student Affiliates:

ALLEN, LOWELL, Dairy Dept., Mich. State College, E. Lansing
 ASSELIN, VIRGIL F., JR., 602 Saginaw St., Norway
 BAGGINS, CHARLES, Dairy Dept., Mich. State College, E. Lansing
 BIRCHMAN, OWEN R., 13653 Ward Ave., Detroit 27
 BLACKFORD, CARL L., Dairy Dept., Mich. State College, E. Lansing
 BLACKFORD, KENNETH, Dairy Dept., Mich. State College, E. Lansing
 BRINKS, KENNETH, Dairy Dept., Mich. State College, E. Lansing
 CHANCE, CHARLES M., Dairy Dept., Mich. State College, E. Lansing
 CHUAN, C. K., Dairy Dept., Mich. State College, E. Lansing
 CLAY, VIRGIL, Dairy Dept., Mich. State College, E. Lansing
 CLELAND, STANLEY, Dairy Dept., Mich. State College, E. Lansing
 ESKIN, MARVAIN, Dairy Dept., Mich. State College, E. Lansing
 FLIPSE, ROBERT, Dairy Dept., Mich. State College, E. Lansing
 FREEMAN, GEORGE A., Dairy Dept., Mich. State College, E. Lansing
 FREEMAN, GEORGE J., Dairy Dept., Mich. State College, E. Lansing
 FREISEN, ALFRED J., Dairy Dept., Mich. State College, E. Lansing
 HATFIELD, ROBERT, Dairy Dept., Mich. State College, E. Lansing
 HERSHBERGER, RUSSELL C., 325 S. Sheldon St., Charlotte
 JOHNSON, F. E., Dairy Dept., Mich. State College, E. Lansing
 JUENGEL, ALLEN VERN, Dairy Dept., Mich. State College, E. Lansing
 KIM, SUNG SUN, Dairy Dept., Mich. State College, E. Lansing
 KNUFF, RICHARD, Dairy Dept., Mich. State College, E. Lansing
 LONGHENRY, GEORGE, 311 N. Nottawa St., Sturgis
 MADRINAN, RAMIRO, Dairy Dept., Mich. State College, E. Lansing
 MCCLELLAN, ELMER, Dairy Dept., Mich. State College, E. Lansing
 NEWLIN, ROBERT, Dairy Dept., Mich. State College, E. Lansing
 SEELEY, WILLIAM, Dairy Dept., Mich. State College, E. Lansing
 SEIBERT, WILLIAM, Dairy Dept., Mich. State College, E. Lansing
 SMITH, WILLIS E., Dairy Dept., Mich. State College, E. Lansing
 STARKS, JACK, Dairy Dept., Mich. State College, E. Lansing
 WEINSTEIN, BERNARD, Dairy Dept., Mich. State College, E. Lansing

WILKOWSKIE, ROGER, Dairy Dept., Mich.
State College, E. Lansing
YOKUM, ARTHUR, Dairy Dept., Mich. State
College, E. Lansing

ZURAKOWSKI, WALTER, Dairy Dept., Mich.
State College, E. Lansing

MINNESOTA

Members:

AHLSTRAND, E. R., 5607 Cambridge St.,
Minneapolis
ALLQUIST, JOHN E., 621 Case Ave., St. Paul
ASTROTH, FRANK B., 1453 Hythe St., St.
Paul 8
BARKER, E. M., Rochester, Dairy Co., Roch-
ester
BARNES, JOHN C., 302 E. Alcott Ave., Fer-
gus Falls
BARTLE, ERVIN, 4517 Washburn Ave., S.,
Minneapolis
BONNEY, RICHARD, 507 S. Cleveland Ave.,
St. Paul 4
BOSWORTH, GROVER, St. Michael
CARLSON, D. T., Willmar
CLARK, DONALD M., Twin City Milk Prod.
Assoc., 1001 Glenwood Ave., N., Minne-
apolis
COMBS, W. B., Dairy Div., University Farm,
St. Paul 8
CONNER, J. K., Lakeville Creamery Co.,
Lakeville
COULTER, S. T., University Farm, St. Paul
FARRAR, ROBERT R., Route 8, St. Paul 10
FINE, N. C., Animal Husbandry Div., Uni-
versity Farm, St. Paul
FITCH, J. B., Dept. of Dairy Husbandry,
University Farm, St. Paul 8
FOURNELLE, H. J., 106 N. Smith Ave., Apt.
340, St. Paul 2
FREDRICKSEN, F. M., 222 3rd St., N.E.,
Faribault
GEIGER, ROBERT, 2274 W. Como Ave., St.
Paul 8
GILMORE, JOHN, Land O'Lakes Farm, R. D.
3, Anoka
GRAF, G. C., Dairy Dept., University Farm,
St. Paul
GULLICKSON, T. W., University Farm, St.
Paul 8
HALVERSON, RALPH, Mora
HANKINSON, CLOYCE L., North American
Creameries, Inc., Paynesville
HANSON, CLEMENTS C., Barnum Creamery
Co., Barnum
HANSON, RAYMOND L., 2082 Como Ave.,
Apt. 7, St. Paul
HARLAND, HERBERT A., Div. of Dairy Hus-
bandry, University Farm, St. Paul 8
HAWKINS, ORVILLE J., 2274 Como Ave., St.
Paul 8
HEALY, HENRY A., Dairy Products Lab.,
2244 Carter Ave., St. Paul 8
HERVEY, MARSHALL C., Div. of Dairy Hus-
bandry, University Farm, St. Paul 8
HODGSON, R. E., Waseca
JENNESS, ROBERT, Univ. of Minn., Div. of
Agr. Biochemistry, University Farm, St.
Paul 8

JEZESKI, JAMES, J., Dept. of Dairy Hus-
bandry, Univ. of Minn., St. Paul
JOHNSON, O. C., 515 State Office Bldg.,
Dairy & Food Dept., St. Paul 1
KETCHAM, WESLEY J., 1125 9½ Ave. S. E.,
Rochester
KOHLER, HENRY, 411 Banning Ave., White
Bear Lake
LARSON, BRUCE L., Dept. of Agr. Biochem-
istry, University Farm, St. Paul
LARSON, GILBERT O., 1407 Randolph St., St.
Paul
LEIGHTON, RAMER, University Farm, St.
Paul 8
LONSDALE, RICHARD E., 3750 N. E. 5th St.,
Minneapolis
MACY, HAROLD, Div. of Dairy Husbandry,
University Farm, St. Paul 8
MATTSON, CARL H., 5729 Pillsbury Ave.,
Minneapolis
MATTSON, H. A., Box 145, Minneapolis
MEYER, CLIFFORD H., Wayzata
MILLER, KENNETH P., Univ. of Minn.,
Grand Rapids
MILLER, WALLACE, New Prague
MODEN, GILMER, 305 Sheridan St., Albert
Lea
NELSON, H. GODFREY, Div. of Public Health,
401 City Hall, Minneapolis 15
NELSON, J. WESLEY, 200 Grain Exchange
Bldg., Minneapolis 15
NUPSON, H. N., Redwood Falls
OLSON, FLOYD C., 435 S. Broadway, Still-
water
OLSON, JOSEPH C., JR., Dept. of Dairy
Bact., University Farm, St. Paul 8
PETERSEN, W. E., University Farm, Dairy
Dept., St. Paul 8
PFEIFER, GEORGE B., 4800 Emerson Ave. S.,
Minneapolis 9
SCARR, DAVID N., West Concord
SEARLES, H. R., Agr. Extension Div., Uni-
versity Farm, St. Paul 8
SEATH, R. W., 104 E. University St., Owa-
tonna
SIPIORA, JULIAN E., Kraft Foods Co., 100
N. 7th St., Minneapolis
SJOWALL, ALFRED L., Maple Island Farm,
Inc., 219 N. Main St., Stillwater
SORENSEN, C. M., 752 Vandalia St., St. Paul
4
SORENSEN, FRANKLIN L., JR., 1967 Grand
Ave., Apt. 203, St. Paul
THOLE, HOWARD W., 1309 E. 4th St., St.
Paul
THOMAS, ELMER, Div. of Dairy Husbandry,
University Farm, St. Paul 8
TJOSVOLD, DALE, 3204 Minnehaha Ave.,
Minneapolis
TRYGGVETH, O. A., 4239 31 Ave. S., Minne-
apolis 6

VACHA, G. A., Room 537, State Office Bldg., St. Paul 1
 WAHENAAR, RAPHAEL, Div. of Dairy Husbandry, University Farm, St. Paul 1
 WAYNE, RALPH W., 2160 Carter Ave., St. Paul 8
 WEIMAR, A. C., 4945 Colfax S., Minneapolis
 WELLS, CLAUDE B., JR., 618 3rd St. S. W., Rochester
 WILSON, JOHN L., Economics Lab., Inc., 914 Guardian Bldg., St. Paul 1
 YOUNG, DONALD E., 4924 4th Ave. S., Minneapolis 9

Student Affiliates:

BOATMAN, PETER T., 1431 Buchanan St. N. E., Minneapolis 13
 BURTNESS, EINAR R., Route 1, Caldonia
 ERICKSON, WALLACE, Route 1, Box 98, Rush City
 GONI, S. K., Div. of Dairy Husbandry, University Farm, St. Paul 1
 GRANT, RALPH S., Mille Lacs County Extension Service, Milaca
 HARTLEY, CLELL, Div. of Dairy Husbandry, University Farm, St. Paul 1
 HEDLUND, LOUIS V., 2060 Carter Ave., St. Paul
 HILL, DONALD L., Div. of Dairy Husbandry, University Farm, St. Paul 1
 HOGLUND, HAROLD, 576 Cromwell Ave., St. Paul 4
 KELKAR, C. N., Div. of Dairy Husbandry, University Farm, St. Paul
 KUBICEK, QUENTIN Waseca
 LAUGHLIN, LAWRENCE R., Box 409, Cambridge
 LEAF, ROBERT, Apples Acres Farm, Route #3, Hastings

LI, LAWRENCE, Div. of Dairy Husbandry, University Farm, St. Paul
 MCCORMICK, PATRICK, 2150 Berkeley St., St. Paul
 MIX, LEW S., Div. of Dairy Husbandry, University Farm, St. Paul
 MORRIS, HOWARD A., Div. of Dairy Husbandry, University Farm, St. Paul
 NELSON, HARVARD G., Div. of Dairy Husbandry, University Farm, St. Paul
 NICKERSON, THOMAS A., Div. of Dairy Husbandry, Univ. of Minnesota, St. Paul
 NIELSEN, ARTHUR J., 1388 Grand Ave., St. Paul 5
 OBERNOLTE, DEAN, McGregor
 OLSON, ARDEN D., 2321 Priscilla St., St. Paul
 OTTO, RAYMOND G., 2848 Buford, St. Paul
 REGER, BERNARD G., Fergus Dairy Coop., Fergus Falls
 REGER, JOSEPH V., 921 Hoyt, St. Paul
 REINARZ, RUSSELL R., 3138 Cedar Ave. S., Minneapolis 7
 RUDNICK, ARTHUR W., JR., Div. of Dairy Husbandry, University Farm, St. Paul
 SIMONSON, LAWRENCE R., 2147 B, University Village, St. Paul
 STONE, HAROLD, 1307 Chelmsford St., St. Paul 8
 STONE, PAUL, 187 Arthur Ave. S. E., Minneapolis 14
 VALO, GEORGE, 2379 Bourne Ave., St. Paul 8
 WEGENER, L. W., School of Agr., University Farm, St. Paul
 YOUNG, GUY S., Marshall
 YOUNG, ROBERT, Canton
 ZELLER, ROLAND, 2137 F Hoyt Ave., University Grove E., St. Paul 8

MISSISSIPPI

Members:

BRACKETT, CHARLES E., Newton
 CONNELLY, H. M., Box 549, Columbus
 COWSERT, W. C., Box 452, State College
 CUNNINGHAM, ROBERT C., 402 S. Montgomery St., Starkville
 FLETCHER, LANE, Animal Husbandry Dept., Miss. State College, State College
 HERZER, F. H., Dept. of Dairying, Miss. State College, State College
 HIGGINS, L. A., Starkville
 HOMER, CLEM, Dairy Dept., State College

LUSK, JOHN W., Box 342, State College
 MITCHELL, S. B., Houston
 WALKER, J. C., Walker Farms, Stoneville
 WATERS, RICHARD E., Starkville

Student Affiliates:

DALEY, CHARLES B., Box 302, State College
 DAVIS, MARVIN E., Box 1199, State College
 FLYNT, WILLIAM C., Box 421, State College
 WORTHINGTON, GLENN A., Route 4, Box 96, Jackson

MISSOURI

Members:

ANDERSON, J. A., 901 S. Rogers St., Springfield
 BRODY, SAMUEL, Dairy Dept., Univ. of Mo., Columbia
 CAINE, RICHARD, Producer's Creamery, Monett
 CASSIDY, B. J., 3110 Gillham Rd., Kansas City 3
 CLONINGER, WILLIAM H., Extension Dairyman, Eckles Hall, Columbia

DAVIS, GLENN, Route 6, Columbia
 DEW, GEORGE, 4451 Raytown Rd., Kansas City 3
 DICKERSON, G. E., Animal Husbandry Dept., Univ. of Mo., 211 S. Schweitzer Hall, Columbia
 EDWARDS, FRED, Producers Creamery Co., Cabool
 FENNER, GEORGE H., Western Dairy & Ice Cream Co., 218 S. 5th St., St. Joseph

FERRAEZ, NICOLAS, JR., 5800 Pershing Ave., St. Louis 12
 GEHRKE, CHARLES W., Dept. of Agr. Chemistry, Univ. of Mo., Columbia
 GHOLSON, JAMES H., 103 Eckles Hall, Univ. of Mo., Columbia
 GRAHAM, W. R., JR., Cerophyl Lab., Inc., P. O. Box 356, Kansas City 10
 HARRIS, RANSOM C., Beatrice Creamery Co., Main & Jules Sts., St. Joseph
 HEINEMANN, BURDET, Producers Creamery Co., P. O. Box 1427, S. S. Station, Springfield
 HENRY, VANCE, 817 College Ave., Columbia
 HERMAN, HARRY A., Dept. of Dairy Husbandry, College of Agr., Columbia
 HUNT, LESLIE L., Pet Milk Company, 1401 Arcade Bldg., St. Louis 1
 ITSCHNER, E. T., Eckles Hall, Univ. of Mo., Columbia
 JOHNSTON, HARVEY L., Route 1, Golden City
 KIBLER, H. H., Dairy Dept., 208 Eckles Hall, Univ. of Mo., Columbia
 KNUDSEN, EDWIN, 814 E. 8th St., Trenton
 KOCHER, DANIEL S., Aines Farm Dairy, 3110 Gillham Rd., Kansas City
 KUMARAN, J. D. S., Dairy Dept., Eckles Hall, Univ. of Mo., Columbia
 LATZER, ROBERT L., 1401 Arcade Bldg., St. Louis 1
 LEACH, CORL A., Miller Bldg., 8th & Broadway, Columbia
 LIDE, B. M., JR., 2001 Chestnut St., St. Louis 3
 MORGAN, ROY, Country Club Dairy Co., 5633 Troost Ave., Kansas City
 POWELL, E. B., Research Farm, Ralston Purina Co., 835 S. 8th St., St. Louis 2
 QUIGLEY, J. V., Chapman Dairy Prod., 1217 Harrison St., Kansas City 10
 RAGSDALE, A. C., Eckles Hall, Univ. of Mo., Columbia
 REGAN, M. J., Dairy Dept., Univ. of Mo., Columbia
 REID, WILLIAM H. E., Dept. of Dairy Husbandry, Univ. of Mo., Columbia
 STALLCUP, ONE TALMADGE, 225 Eckles Hall, Columbia
 STEPHENS, A. F., 4929 McPherson, St. Louis
 THOMPSON, J. C., Ralston-Purina Co., 835 S. 8th St., St. Louis 2

TURNER, CHARLES W., Dept of Dairy Husbandry, Univ. of Mo., Columbia
 VARNEY, BRUCE, 328 Board of Trade Bldg., 127 W. 10th St., Kansas City 6
 WEAR, JOHN M., JR., Purina Research Farm, Gray Summit

Student Affiliates:

AGEE, CALVIN B., 1528 Locust St., St. Louis 3
 ANDERSON, DONALD, 201 Eckles Hall, Columbia
 BASSNETT, ROBERT, 123 Eckles Hall, Columbia
 BLATTNER, ROBERT, Route 1, Columbia
 COBBLE, JAMES, 127 Eckles Hall, Columbia
 CONRAD, A. B., 809 College Ave., Columbia
 DAY, JOHN P., 216 N. Moffet Ave., Joplin
 DICKENSHEET, MAURICE, 814 Virginia Ave., Columbia
 EDMONDSON, JUSTUS H., 127 Eckles Hall, Columbia
 HART, WALLACE, Box 594, Columbia
 HARTLEY, J. L., 1 Observatory Hill, Columbia
 KAMAL, SYED, 214 Eckles Hall, Columbia
 KAUFFMAN, EUGENE, 307 College, Columbia
 LABEN, ROBERT C., 127 Eckles Hall, Columbia
 MEREDITH, WILLIAM H., 1507 Bouchelle, Columbia
 MERILAN, CHARLES P., 201 Eckles Hall, Columbia
 MONROE, ROBERT A., 217 Eckles Hall, Columbia
 PIPES, GAYLE W., 217 Eckles Hall, Columbia
 PORTER, VERNON L., 51 N. Park St., Cape Girardeau
 PRISKLEY, GLEN, 201 Eckles Hall, Columbia
 RECTOR, GLEN, R. D. 1, Rush Hill
 RISK, MARION A., 607 W. Ash, Columbia
 ROARK, DONALD B., 225 Eckles Hall, Columbia
 SCHWINKE, ARLEN, 802 Virginia, Columbia
 TALLMAN, KENNETH L., 223 Eckles Hall, Columbia
 WALL, CHESTER, 1516 Ross, Columbia
 WARDLOW, VICTOR, 3104 Benton Blvd., Kansas City
 WEBER, GILBERT, 720 Missouri, Columbia
 WERTH, HOWARD, 201 Eckles Hall, Columbia

MONTANA

Members:

BRATTON, EDWARD, Ronan
 BRENCE, JOHN L., Dept. of Dairy Industry, Bozeman
 GOBLE, HAROLD, Jersey Dairy, Bozeman
 HARRISBERGER, WARREN, P. O. Box 1381, Great Falls
 KEYES, EVERETT A., Dept. of Dairy Industry, Mont. State College, Bozeman
 KOPLAND, D. V., Huntley Field Station, Huntley
 MACIVER, KENNETH S., 402 West Cleveland, Bozeman

MITCHELL, FERGUS G., Ayrshire Dairy, Great Falls
 NELSON, J. A., Dept. of Dairy Industry, Mont. State College, Bozeman
 ROSENKAT, FRED, 425 1st Ave. S.W., Great Falls
 SLACK, RANSOM O., Gold Medal Dairies, Missoula
 TRETSVEN, J. O., Dairy Specialist, Mont. State College, Bozeman

Student Affiliates:

BRATTON, DONALD, Dairy Industry Dept., Mont. State College, Bozeman

BURGER, MARIE, Dairy Industry Dept., Mont. State College, Bozeman
 CLARK, RALPH, Dairy Industry Dept., Mont. State College, Bozeman
 CRUMBAKER, HOWARD, Dairy Industry Dept., Mont. State College, Bozeman
 GANDER, J. W., Dairy Industry Dept., Mont. State College, Bozeman
 HAWKINS, W. W., JR., Dairy Industry Dept., Mont. State College, Bozeman
 HUANG, W. Y., Dairy Industry Dept., Mont. State College, Bozeman
 MCHUGER, E. P., Dairy Industry Dept., Mont. State College, Bozeman

McMURRAY, J. A., Dairy Industry Dept., Mont. State College, Bozeman
 NELSON, J. H., 1110 S. 5th, Bozeman
 PEACE, EARL, Dairy Industry Dept., Mont. State College, Bozeman
 REHBERG, WALLACE, Dairy Industry Dept., Mont. State College, Bozeman
 SMITH, ERVIN, Dairy Industry Dept., Mont. State College, Bozeman
 STOCKING, JOHN A., Rural Route, Huntley
 TURNER, GORDON, Dairy Industry Dept., Mont. State College, Bozeman

NEBRASKA

Members:

CROWE, L. K., Dairy Husbandry Dept., Univ. of Neb., Lincoln
 DAVIS, H. P., Dairy Dept., College of Agr., Lincoln
 DAY, R. E., Allied Mills, Inc., Omaha
 DEANE, DARRELL D., Dept. of Dairy Husbandry, Univ. of Neb., Lincoln
 DOWNS, P. A., Dept. of Dairy Husbandry, Univ. of Neb., Lincoln
 FARR, R. H., 5636 Oak St., Omaha
 FOSSLAND, ROBERT G., Dairy Husbandry Dept., Univ. of Neb., Lincoln
 GERRISH, F. S., 609 Redick Tower, Omaha 2
 HATHAWAY, IRWIN L., Dept. of Dairy Husbandry, Univ. of Neb., Lincoln
 HOWE, D. K., Fairmont Foods Co., Omaha

NIBLER, C. W., Dairy Bldg., College of Agr., Lincoln
 RUMERY, MYRON, Univ. of Neb. Expt. Station, North Platt
 SANDERS, CHRIS H., Univ. of Neb., Dairy Dept., Lincoln
 SCHULTZE, ANDREW B., Dept. of Dairy Husbandry, Univ. of Neb., Lincoln
 TEMPLETON, HUGH L., 6125 Florence Blvd., Omaha 11
 WASSON, ABELL J., 2413 Leavenworth St., Omaha

Student Affiliates:

BAKER, FOREST, 1221 N. 37th St., Lincoln
 COLE, PHILLIP, 3905 Dudley St., Lincoln

NEVADA

Members:

HEADLEY, F. B., Dept. of Farm Div., Univ. of Nev., Reno

NEW HAMPSHIRE

Members:

AMODON, WINTHROP R., c/o H.H.B.A., Bte. 4, Concord
 BEAUTES, LOUIS J., R. D. 1, West Canaan
 BOYNTON, HILTON, Extension Dairyman, Univ. of N. H., Durham
 CUMMINGS, C. M., American Guernsey Cattle Club, Peterborough
 FARRAR, PRESCOTT S., Div. of Food & Chemistry, State Dept. of Health, Concord

KEENER, HARRY A., Dept. of Dairy Husbandry, Univ. of N. H., Durham
 MOORE, H. C., Dairy Dept., Univ. of N. H., Durham
 MORROW, K. S., Dairy Husbandry Dept., Univ. of N. H., Durham
 OLSSON, G. B., Dairy Dept., Univ. of N. H., Durham
 PIKE, ADNA B., Dept. of Agr., Concord

NEW JERSEY

Members:

BARTLETT, J. W., Dairy Dept., N. J. Agr. College, New Brunswick
 BAUFENFEIND, J. C., Hoffman-La Roche, Inc., Nutley 10
 BECKER, HENRY, Livingston Ave., Roseland
 BENDER, CARL B., Dept. of Dairy Husbandry, N. J. Agr. Expt. Station, New Brunswick
 BERGGREN, RUTH E. L., Kind & Knox Gelatin Co., Head of No. 5th St., Camden
 BERGLINER, VICTOR R., Ortho Pharmaceutical Corp., Raritan

BISHOP, JOHN V., Columbus
 BOROWSKY, HARRY H., Onyx Oil & Chemical Co., Warren and Morris St., Jersey City
 BRYAN, JOHN S., c/o Walker-Gordon Laboratory Co., Plainsboro
 CLARK, ROBERT I., Apt. 2B-30A, Meadowbrook Village, Plainfield
 COCHRAN, RICHARD F., 560 Goffe Rd., Wyckoff
 CONN, RALPH I., 4 Carpenter Terrace, Belleville

DOUGHTY, RICHARD S., 34 Hillside Ave.,
Chatham
FRANCISCO, CARL, c/o Middlebrook Farm,
Route 1, Asbury Park
GAREY, COREY C., JR., Dairy Research Sta-
tion, Sussex
GREEN, D. F., 125 Thomas St., Cranford
HERROLD, JOHN, Library, National Oil
Products Co., Harrison
JOHNSTON, JAMES E., Dairy Research Farm,
Sussex
KEENEY, MARK H., Cedar Grove
KOLESOFF, VALETIAN F., R. D. 1, Farm-
ingdale
LEAR, SAMUEL A., Dairy Husbandry Dept.,
Rutgers Univ., New Brunswick
LEEDER, JOSEPH, Dept. of Dairy Industry,
Rutgers Univ., New Brunswick
LEVOWITZ, DAVID, N. J. Dairy Lab., 226
Eastern Ave., New Brunswick
LITTLE, LAWRENCE, E. F. Drew & Co., Inc.,
416 Division St., Boonton
MATHER, R. E., Dairy Research Station,
Sussex
MCCAN, J. C., 246 Grant Ave., Highland
Park
MIXNER, JOHN P., Dairy Research Station,
Sussex
MOSS, J. R., 709 Mountain Ave., Westfield
NAIR, JOHN H., Continental Foods, Inc.,
Hoboken
PERRY, E. J., N. J. Agr. College, New
Brunswick
PFAU, KARL O., Dairy Research Station,
Sussex
REECE, RALPH P., Dept. of Dairy Hus-
bandry, N. J. Agr. Expt. Station, New
Brunswick
SCHEIDENHELM, E. C., Dairy Dept., Rutgers
Univ., New Brunswick
SHUART, EDMUND C., N. J. Agr. Expt. Sta-
tion, Sussex

TITUS, HARRY W., Lime Crest Research
Laboratory, R. D. 1, Newton
TREGURTHA, JAMES D., Newark Milk &
Cream Co., 26 Bridge St., Newark
VAHLTEICH, H. W., Best Foods Co., 99
Ave. A, Bayonne
WALDRON, H. R., Califon
WELSH, MARK, D.V.M., 239 Beechwood Rd.,
Ridgewood
WIGGIN, STANLEY, R. F. D. 1, Newton

Student Affiliates:

DEMINTZ, PETER E., 215 3rd St., Palisades
Park
FOSTER, HERBERT G., JR., Dairy Dept., Rut-
gers Univ., New Brunswick
GLASS, NORMAN R., 325 Clearfield Ave.,
Trenton
JOHNSON, ROBERT, 414 Bender Ave., Roselle
Park
KELLEY, EDWARD, 729 Madison Ave., Eliza-
beth
KLERK, JANET, Gibbons 36, N. J. College of
Women, Rutgers, New Brunswick
LEISSLER, CHARLES, 3rd, 2 Palmer St.,
Bloomfield
MCCORMICK, KENNETH, JR., 71 Maolis Ave.,
Bloomfield
MILLER, CHARLES, 627 Floral Ave., Eliza-
beth
NOTTER, WILLIAM, Village Rd., New Vernon
PEKERIS, ARTHUR, 9 Handy St., New Bruns-
wick
POTTER, ALBERT A., c/o De Neef, Branch-
ville
SCHERHOLZ, JOHN, 36 Lawrence Ave., W.
Orango
STAHLMAN, CLARENCE, c/o H. Weiss, 475
Belmont Ave., Newark 8
TREGURTHA, JAMES D., 40 Claremont Ave.,
Bloomfield
VREELAND, SANFORD, Allamuchy Rd., Hack-
ettstown

NEW MEXICO

Members:

JENTGEN, WALTER F., Price's Creamery,
Portales
KEEVES, CALVIN B., Dairy Dept., N. M. A.
& M. College, State College
TRETSEN, WAYNE, Dairy Dept., Box 186,
State College

Student Affiliates:

BIRCHALL, RICHARD P., Box 501, State Col-
lege
DIXON, JIM, Gen. Delivery, Taos
LOPEZ, ELIAS B., 634 W. Court, Las Crecas
POTTS, FRED, Gen. Delivery, Taos
SCARBOROUGH, EL RITO
SCHWARTZ, DANIEL O., Gen. Delivery, State
College

NEW YORK

Members:

ALBRECHTSEN, RAYMOND, Cornell Univ., Dept.
of Animal Husbandry, Ithaca
AYRES, W. E., 115 Ithaca Rd., Ithaca
BARBER, FRANKLIN W., National Dairy Re-
search Lab., Oakdale, L. I.
BARSCH, WALTER C., Pioneer Ice Cream
Division, 205 E. 24th St., New York 10
BAYER, A. H., Director of Research & Prod-
uct, General Ice Cream Corp., 710 East-
ern Ave., Schenectady

BEATY, ANNABEL, National Dairy Research
Labs., Inc., Oakdale, L. I.
BEGLEY, KEMP, R. D. 2, Honeoye Falls
BELL, LEWIS S., Interlaken
BENNETT, S. R., National Milk Sugar Co.,
Div. of Borden Co., Bainbridge
BLOCK, RICHARD J., 15 Cooper Rd., Sears-
dale
BOTWINIK, LEONARD, 1288 E. 10th St.,
Brooklyn 30

- BOWLING, G. A., Strathglass Farm, Port Chester
- BRADY, C. G., Animal Husbandry Dept., Cornell Univ., Ithaca
- BRADWAY, ELIZABETH M., Borden Laboratory, 600 N. Franklin St., Syracuse 4
- BRATTON, ROBERT W., Dept. of Animal Husbandry, Cornell Univ., Ithaca
- BREED, ROBERT S., 6 Sunset Drive, Geneva
- BROWNELL, S. J., Dept. of Animal Husbandry, Cornell Univ., Ithaca
- BROWNING, R. A., Park & Pollard Co., 356 Hertel St., Buffalo
- BURKE, JAMES D., Route 4, Ithaca
- BURRELL, LOOMIS, Cherry-Burrell Corp., Little Falls
- CARPENTER, HION, 603 N. Tioga St., Ithaca
- CARTER, WILMOT, Wing Hall, Cornell Univ., Ithaca
- CHALMERS, ARTHUR A., II, 439 Guy Park Ave., Amsterdam
- CLAPP, ELMER E., JR., New York State Agr. & Tech. Institute, Canton
- CLARIN, DAVID X., Oakite Products, Inc., 22 Thames St., New York 6
- CLEVELAND, CHARLES, Middletown Milk & Cream Co., Slate Hill
- COHEN, HAROLD, 28 Oakman St., Rochester 5
- COHEN, ISAAC, 76-01 Myrtle Ave., Brooklyn 27
- CORDES, WILLIAM A., Sealtest, Inc., 230 Park Ave., New York 17
- CRANDALL, W. T., N. Y. State College of Agr., Cornell Univ., Ithaca
- DAHLBERG, A. C., Cornell Univ. Ithaca
- DAVISON, R. O., 31 Nassau St., New York 5
- DETWILER, B. H., 110 Hudson St., New York 13
- DUBIN, ABRAHAM J., 336 West End Ave., New York 23
- EASTWOOD, H. S., DeLaval Sales & Service, Inc., Poughkeepsie
- FISH, MILTON H., South Edmeston
- FISHMAN, ROY H., Standard Brands, Inc., 595 Madison Ave., New York 22
- FOOTE, ROBERT H., Dept. of Animal Husbandry, Cornell Univ., Ithaca
- FREIDMAN, AARON, 327 Central Park West, Apt. 5F, New York 25
- GARMAN, VICTOR C., 1 Waverly Place, Albany 3
- GAVIN, JOSEPH S., Gavin Dairy Laboratory, 200 Stockbridge, Buffalo 15
- GERNCI, JOSEPH J., 63 Lux St., Rochester 5
- GRAHAM, RUSSELL A., 146 Brampton Rd., Syracuse
- GREZE, J. P., Oakite Prod., Inc., 22 Thames St., New York 6
- GURDIAN, MAX, 114 Cobb St., Ithaca
- GUTHRIE, E. S., Cornell Univ., Ithaca
- HALLETT, RAY, Sodus Creamery Corp., Wolcott
- HAMLIN, FRANK H., Papee Machine Co., Shortsville
- HANSEL, WILLIAM, Animal Nutrition Lab., Stocking Hall, Cornell Univ., Ithaca
- HARDING, H. G., National Dairy Prod., Research Lab., Oakdale, L. I.
- HARMON, E. M., National Dairy Council, 437 5th Ave., New York 16
- HARRIS, THEODORE WILLIAM, Hinman Milking Machine Co., Inc., Oneida
- HEIMAN, VICTOR, Kasco Mills, Inc., Waverly
- HENDERSON, CHARLES R., Animal Husbandry Dept., Cornell Univ., Ithaca
- HENING, J. C., New York Agr. Expt. Station, Geneva
- HERRINGTON, B. L., Dept. of Dairy Industry, Cornell Univ., Ithaca
- HERRO, ALEX C., National Dairy Research Lab., Oakdale, L. I.
- HESSLE, FRED H., Coop. G. L. F., Mills Division, Box 973, Buffalo 5
- HILEMAN, J. L., Dairymen's League, 810 Burnett, Syracuse 3
- HILKER, LUTHER D., National Dairy Research Lab., Inc., Oakdale, L. I.
- HOLLAND, ROBERT F., Dairy Dept., Cornell Univ., Ithaca
- HOPSON, GEORGE H., South Rd., Millbrook
- HOYNAK, P. X., Refined Syrups & Sugars, Inc., Yonkers
- JANSEN, J. F., 1 Union St., Oneonta
- JOHNSON, ARNOLD H., National Dairy Research Lab., Inc., P. O. Box 97, Oakdale, L. I.
- JOHNSON, MAURICE A., 103 E. York St., Ithaca
- JONES, FRANK L. SEYMOUR, c/o Borden Co., 350 Madison Ave., New York 17
- JUDKINS, H. F., 230 Park Ave., New York 17
- KERN, CLYDE L., 11 West 42nd St., 21st Floor, New York 18
- KIEDA, ADAM, Treadwell
- KIESSEL, GEORGE K., 18 Highland Terrace, Manhasset, L. I.
- KIMBLE, THOMAS A., Dairy Husbandry Dept., Cornell Univ., Ithaca
- KISSEN, BENJAMEN, 30 Church St., New York 7
- KNAYSI, GEORGE, Dairy Industry Bldg., Cornell Univ., Ithaca
- KOERVER, C., Pioneer Ice Cream Division, 205 E. 24th St., New York 10
- KOSIKOVSKY, FRANK V., Dairy Bldg., Cornell Univ., Ithaca
- KRATZER, CARL R., Borden Company, Arcade
- KRAVITZ, MAX, 133 Gorham St., Cannondaugua
- KRUKOVSKY, V. N., Dept. of Dairy Industry, Cornell Univ., Ithaca
- LAMB, LELAND W., 213 E. Seneca St., Ithaca
- LARSON, C. W., Tudor Plaza Apts., 741 W. Ferry St., Buffalo 9
- LAZARUS, N. E., 266 Bryant St., Buffalo 9
- LEBER, HENRY, 810 Burnett, Syracuse
- LEHMKUHL, HENRY, 73 Howell St., Rochester 7
- LIST, W. H., Ass'n of Ice Cream Mfgs., Hotel Pennsylvania, 7th Ave. & 34th St., New York 1
- LOOSLI, J. K., Laboratory of Animal Nutrition, Cornell Univ., Ithaca

- LUNDSTEDT, ERIK, 16 Parkway, Goshen
 MARCH, RICHARD, The Knoll, c/o A. H. Treman, Ithaca
 MARCUSSEN, WILLIAM H., Borden Co., 350 Madison Ave., New York 17
 MARQUARDT, J. C., 14 Maxwell Ave., Geneva
 MASTERS, FRANK, 213 E. Seneca, Ithaca
 MASUROVSKY, B. I., 2784 Morris Ave., Bronx 58
 MAYNARD, L. A., Lange Hall, Cornell Univ., Ithaca
 MCCADAM, L. W., 408 Ford St., Ogdensburg
 MCCHESENEY, E. R., Fairmont Creamery Co., Buffalo
 MONRAD, KARL J., Chr. Hansen's Lab., Inc., Little Falls
 MOOK, D. E., 32 Secor Rd., Scarsdale
 MORRISON, F. B., Dept. of Animal Husbandry, Cornell Univ., Ithaca
 MUSER, SIDNEY, 250 West 57th St., New York 19
 MYERS, E. M., New York School of Agr., Alfred
 MYERS, R. P., National Dairy Research Lab., Oakdale, L. I.
 NAYLOR, H. B., Dairy Industry Dept., Cornell Univ., Ithaca
 NEWMAN, P. E., Beacon Milling Co., Cayuga
 NEZVESKY, LOUIS, 508 Highland Rd., Ithaca
 NORRIS, L. C., Rice Hall, Cornell Univ., Ithaca
 NORTH, CHARLES E., 23 E. 26th St., New York 10
 PALEY, CHARLES, Certified Laboratories, Inc., 19 Hudson St., New York 13
 PETERSON, JOHN B., Dept. of Animal Husbandry, Cornell Univ., Ithaca
 PLETCHER, F. H., L. I. Agr. & Tech. Institute, Farmingdale
 POE, JOHN W., 442 East Veteran's Place, Cornell Univ., Ithaca
 POWERS, A. J., Borden Farm Products Co., Inc., Laboratory Dept., 90 3rd Ave., Brooklyn
 QUENCER, ARTHUR B., 620 12th Ave., New York 19
 RANDALL, WALDO W., Mount Sinai
 REID, J. THOMAS, Dept. of Animal Husbandry, Cornell Univ., Ithaca
 RICHARDS, CLYDE R., Wing Hall, Cornell Univ., Ithaca
 RIGGS, L. K., National Dairy Research Lab., Inc., Oakdale, L. I.
 ROBERTSON, A. H., Director of State Food Lab., New York State Dept. of Agr. & Mkts., State Office Bldg., Albany
 ROLLINS, NORMAN W., Wing Hall, Cornell Univ., Ithaca
 ROSS, HARRY ALBERT, Bureau of Economics, The Borden Co., 350 Madison Ave., New York 17
 SCHAEFER, O. G., Room 2304, 420 Lexington Ave., New York 17
 SCHNEIDER, KILIAN, Arkport
 SCHULZ, L. H., Animal Husbandry Dept., Cornell Univ., Ithaca
 SEMLER, EDWIN L., Churchville
 SHAUL, JOHN, Forest Home, c/o Prof. King, Ithaca
 SHERMAN, J. M., Dairy Industry Bldg., Ithaca
 SHIPE, W. F., Dairy Husbandry Dept., Cornell Univ., Ithaca
 SHIVELY, BRUCE E., Sheffield Farms Co., 424 W. 57th St., New York 19
 SPALDING, ROBERT W., Dept. of Animal Husbandry, Ithaca
 SPEAKS, CHARLES, Milk Industry Foundation, Chrysler Bldg., New York 17
 STARK, C. N., Cornell Univ., Dairy Bldg., Ithaca
 STOVER, OSCAR H., Hubbard Rd., East Aurora
 SUPPLEE, G. C., c/o Supplee Research Corp., Bainbridge
 SWANSON, WILLIAM J., Distillation Products, Inc., 755 Ridge Rd. West., Rochester 13
 TAILBY, G. W., Animal Husbandry Dept., Cornell Univ., Ithaca
 TAUB, JOEL, 40 E. 17th St., Brooklyn 26
 THOMPSON, E. C., 1792 E. 22nd St., Brooklyn
 TIEDMAN, WALTER VON DOHLEN, 10 Lincoln Ave., Delmar
 TJARKS, E. A., 52 Main St., Port Washington
 TREBILCOCK, ROY J., Corn Prod. Sale, 17 Battery Place, New York
 TRIMBERGER, GEORGE W., Animal Husbandry Dept., Cornell Univ., Ithaca
 TURK, KENNETH L., Dept. of Animal Husbandry, Cornell Univ., Ithaca
 VORPERIAN, JOHN H., 117-01 Park Lane S., Kew Gardens, L. I.
 WALLACE, HAROLD D., 321 Dryden Rd., Ithaca
 WALLIS, G. C., Special Prod. Dept., Standard Brands, Inc., 595 Madison Ave., New York 22
 WASHBON, W. E., 97 Kent Blvd., Salamanca
 WATERHOUSE, T. P., JR., Bellevue Dairy, 729 Broadway, Schenectady
 WEBSTER, H. G., c/o Dairyalea Milk Prod., Inc., Wurz Ave., Utica
 WEINBERGER, ROBERT I., 951 Carroll St., Brooklyn
 WELKER, RALPH M., R. D. 4, Ithaca
 WENTWORTH, W. A., c/o The Borden Company, 350 Madison Ave., New York 17
 WEST, GEORGE A., Rochester Health Bureau, 44 Marshall St., Rochester 2
 WHITAKER, R., National Dairy Research Lab., Oakdale, L. I.
 WHITE, JAMES C., Dairy Bldg., Cornell Univ., Ithaca
 WILLIAMS, ALEXANDER W., Room 1701, 350 Madison Ave., New York 17
 WILLMAN, H. A., Animal Husbandry Dept., Ithaca
 WINNING, ROSS J., 524 W. 57th St., New York 19
 YOUNG, HAROLD, National Dairy Research Lab., Oakdale, L. I.

Student Affiliates:

APIELLA, JOSEPH N., 72 Woods Ave Roosevelt
 BUZZELLI, FRANK G., 527 19th St., Niagara
 Falls
 CACIOFFO, JOSEPH L., 7106 Ingram St., For-
 est Hills
 DANIELS, R. W., 103 Veterans Place, Ithaca
 DAS, B. C., 210 Li Den Ave., Ithaca
 DUNN, HENRY O., 204 Klinewoods Rd.,
 Ithaca
 FLEISCHMAN, FREDERICK F., JR., 127 N.
 Quarry St., Ithaca
 GIBBONS, A. P., 136 Dryden Rd., Ithaca
 GRANLACK, KEN C., 405 Onderdenk Ave.,
 Brooklyn 27
 GREEN, WILLIAM, III, 1701 Union St., Sche-
 nectady 8
 HAGBERG, E. C., National Dairy Research
 Lab., Inc., Oakdale, L. I.
 HARDISON, AUREL, Dept. of Animal Hus-
 bandry, Cornell Univ., Ithaca
 HEDIN, CARL A., 670 Tower Rd., Ithaca
 HEFFERNAN, F. P., Fabius
 HOVEY, GEORGE A., 9 Woodland Place,
 Ithaca

JENKE, STANTON F., Ravana
 JORDAN, WILLIAM K., 708 E. Buffalo St.,
 Ithaca
 KAMANI, B. T., Dept. of Dairy Industry,
 Cornell Univ., Ithaca
 MARLOTT, DONALD DADE, 16 Randall St.,
 Cortland
 MCPHEE, EDWARD, 132 E. Maple Ave., E.
 Rochester
 MUSGRAVE, STANLEY, Animal Husbandry
 Dept., Cornell Univ., Ithaca
 SILVERMAN, GERALD, 191 Malta St., Brook-
 lyn
 THEOKAS, DIONISIOS A., 671 E. Tower Rd.,
 Ithaca
 TOMAINO, FRANK A., 634 Elizabeth St.,
 Utica
 TOWLE, EDMUND J., JR., 311 S. Manning
 Blvd., Albany
 WAGNER, PAUL F., 527 Northland Ave.,
 Buffalo
 WARNER, RICHARD, Lab. of Animal Nutri-
 tion, Cornell Univ., Ithaca
 WEDEEN, MARVIN M., 829 Locust Ave.,
 Schenectady

NEW ZEALAND

Members:

MCDOWALL, F. H., Dairy Research Institute,
 Box 602, Palmerton North

NORTH CAROLINA

Members:

AREY, J. A., State College Station, Raleigh
 BRACKETT, CHARLES E., c/o Kraft Foods
 Co., W. Jefferson
 CARITHERS, R. L., 352 Westwood Dr.,
 Statesville
 CLEVENGER, W. L., Dairy Dept., State Col-
 lege, Raleigh
 CLINE, G. R., Box 29, Chapel Hill
 COLWARD, DEAN W., Dairy Husbandry Dept.,
 N. C. State College, Raleigh
 FAIRES, E. W., Coastal Plain Station, Wil-
 lard
 GOFORTH, HOWARD J., Coble Dairy Products,
 Lexington
 GRINNELS, C. D., Univ. of N. C., Raleigh
 HILTON, J. H., N. C. College of Agr. Ra-
 leigh
 KENNEDY W. L., A & T College, Greensboro
 KIMREY, A. C., Extension Dairyman, N. C.
 State College, Raleigh
 LUCAS, HENRY L., JR., Institution of Sta-
 tistics, N. C. State College, Raleigh

ORDERS, W. CARL, 2711 Rosedale Ave. 1,
 Raleigh
 ROBERTS, W. M., Dept. of Animal Industry,
 N. C. State College, Raleigh
 RUFFNER, R. H., Dept. of Animal Hus-
 bandry, State College Station, Raleigh
 SPECK, MARVIN L., Dept. of Animal Indus-
 try, N. C. State College, Raleigh
 WAUGH, B. K., Dept. of Animal Industry,
 N. C. State College, Raleigh
 WISE, GEORGE H., Dept. of Animal Indus-
 try, N. C. State College, Raleigh
 WYNN, ROBERT L., Box 68, A & T College,
 Greensboro

Student Affiliates:

BOSWELL, JOHN I., Dairy Dept., 204 Polk
 Hall, N. C. State College, Raleigh
 NICHOLSON, WALLER S., JR., Dairy Dept.,
 204 Polk Hall, N. C. State College, Ra-
 leigh
 ROBERTS, W. M., Animal Industry Dept.,
 N. C. State College, Raleigh
 SHOENBERGER, 613 Marsh Rd., Charlotte

NORTH DAKOTA

Members:

BECK, LYLE D., Dairy Husbandry, N.D.A.C.,
 Fargo
 GAALAAS, R. F., U. S. Dairy Station, Man-
 dan
 JENSEN, C., Dairy Husbandry Dept., N.D.
 A.C., Fargo

KELLEY, ROBERT O., Dairy Dept., N. Dak.
 Agr. College, Fargo
 OLSON, CLARENCE C., N.D.A.C., Dairy Divi-
 sion, Fargo
 STONE, EDWARD JAMES, Dairy Dept. N.D.
 A.C., State College Station, Fargo

WOODY, CLYDE DALE, Madison Silos, Box
1941, Jamestown

Student Affiliates:

MARTIN, N.A.D.C., Fargo

OHIO

Members:

ALEXANDER, C. B., Akron Pure Milk Co.,
273 Bowery St., Akron 8
ALMY, E. F., Dept. of Agr. Biochemistry,
Ohio State Univ., Columbus 10
ALTER, JOHN R., 2246 Barrymore Ave., Co-
lumbus 3
ANDERSON, O. E., Ohio Dairy Products
Ass'n., 5 E. Long St., Columbus 15
ARMSTRONG, T. V., 44 E. Frambes Ave.,
Columbus 1
BABCOCK, WAYNE, 945 Berdan Ave., Toledo
BACHTEL, R. T., c/o Borden Co., Noaker
Division, 1112 E. Tusc St., Box 790, Can-
ton 1
BAISLER, WALTER R., 642 McClurg Rd.,
Youngstown 5
BAKER, NEIL R., 239 W. Spring St., Co-
lumbus 15
BAUR, L. S., 653 Glenmont Ave., Colum-
bus 2
BEARDSLEY, JOHN P., American Jersey Cat-
tle Club, 107 N. 6th St., Columbus 15
BECK, ALFRED J., 258 Grandview Ave.,
Wadsworth
BELL, HENRY, Route 1, Peninsula
BENNETT, C. L., c/o Matthews-Frechting
Dairy Co., 2363 St. James Ave., Cincin-
nati 6
BERG, E. K., R. R. 1, Copley
BICHSEL, WILLIAM J., 139 2nd St., N.W.,
New Philadelphia
BLACK, ROBERT, North Canton Dairies, Inc.,
North Canton
BLACKMAN, C. L., Ohio State Univ., Co-
lumbus 10
BRAKEL, WILLIAM J., Plumb Hall, Ohio
State Univ., Columbus 10
BURGWALD, L. H., Dept. of Dairy Technol-
ogy, Ohio State Univ., Columbus 10
BURKEY, L. C., Creamery Package Mfg. Co.,
500 Broadway, Toledo
CAMPBELL, E. E., White Mountain Cream-
ery Co., 124 W. Wayne St., Lima
CAMPBELL, F. M., c/o The Andalusia Dairy
Co., Salem
CAVANAUGH, J. F., American Jersey Cattle
Club, Columbus 15
CHARLES, DONALD A., 1132 Piermont Rd.,
Cleveland 21
CHRYSLER, L. H., 322 S. Roys Ave., Co-
lumbus
COMPTON, ERNEST, Isaly's, Inc., 2800 N.
High St., Columbus 2
CORN, ORVILLE, Meyer Dairy Products, 3051
E. 63rd St., Cleveland
DANIELS, PAUL, Flordell Farms, Kenton
DENLINGER, H. E., 809 Quimby Ave., Woos-
ter
DENNIS, J. DREXEL, 1441 Plain Ave. N.E.,
Canton 4
DIEHL, M. W., 275 Pleasant Valley Rd.,
Cleveland 9

DISSLY, L. P., 3068 W. 106th St., Cleve-
land 11
DODD, D. R., 201 Townshend Hall, Ohio
State Univ., Columbus 10
DRAIN, H. D., Borden's Dairy, 934 Grant
St., Akron
DRAKE, MAX, Route 4, Tiffin
DEUSENDAHL, L. G., 1311 Arlington Rd.,
Lakewood 7
ELLSWORTH, P. R., 592 E. Beechwood, Co-
lumbus
ELY, FORDYCE, Dept. of Dairy Husbandry,
Ohio State Univ., Columbus 10
ERB, J. HOFFMAN, 1697 Berkshire Rd., Co-
lumbus 12
ESHELMAN, RUSSELL H., Dr. Hess & Clark,
Inc., Ashland
FORBES, BENJAMIN F., Brecksville
FROHRING, PAUL R., Chagrin Falls
FROHRING, W. O., Box 232, Chagrin Falls
GARRETT, O. F., c/o M & R Dietetic Lab.,
Inc., 585 Cleveland Ave., Columbus 16
GEBHARDT, H. T., Route 5, Marysville
GILMORE, LESTER O., Dairy Husbandry
Dept., Plumb Hall, Ohio State Univ., Co-
lumbus 10
GINGERY, ROY, 3320 E. 139th St., Cleve-
land 20
GOULD, IRA A., Dairy Technology Dept.,
Ohio State Univ., Columbus 10
GREINER, FRED J., 44 W. Torrence Rd., Co-
lumbus
HAMILTON, ROBERT, Willard Dairy, Willard
HAMILTON, T. KLINE, Diamond Milk Pro-
ducts Inc., 315 Graham St., Columbus 3
HARMAN, THOMAS, 325 S. Court St., Circle-
ville
HAYDEN, C. C., Ohio Agr. Expt. Station,
Wooster
HELWIG, J. H., College of Vet. Medicine,
Ohio State Univ., Columbus 10
HIBBS, JOHN W., Ohio Agr. Expt. Station,
Wooster
HOLESKI, CASHMERE, Oak Hill Rd., Route 1,
Peninsula
HOLESKI, FRANK, R. F. D. 1, Peninsula
HOLM, WARD, c/o Columbus Milk Distribu-
tors Assoc., 35 E. Gay St., Columbus 15
JEWELL, R. M., 1103 E. Vine St., Mt. Ver-
non
JOHNSTON, FLOYD, American Jersey Cattle
Club, 107 N. 6th St., Columbus 15
JOHNSTON, W. L., Westerville Creamery Co.,
Westerville
KAESER, HAROLD, 4871 Olentangy Rd., Co-
lumbus 2
KELLOGG, RICHARD H., 1224 Alton Darby
Rd., Columbus 4
KENNEDY, R. N., The Borden Co., 165 N.
Washington Ave., Columbus 16
KESSELRING, L. M., Kesseling Dairy, 1559
Triplett Blvd., Akron 6

- KNOOP, C. E., Ohio Agr. Expt. Station, Wooster
- KOCH, IRVING O., Bordens Dairy & Ice Cream Co., Columbus 16
- KOLAR, RAYMOND E., 702 Broadway, Piqua
- KRAUSS, WILLIAM E., Ohio Agr. Expt. Station, Wooster
- KRILL, W. R., Veterinary Clinic, Ohio State Univ., Columbus 10
- LENGACHER, JOHN, Route 1, Dundee
- LOWE, ROBERT W., 940 Washington Ave., Cuyahoga Falls
- LUDWICK, THOMAS M., Dairy Husbandry Dept., Ohio State Univ., Columbus 10
- LYMAN, J. F., Dept. of Agr. Chemistry, Ohio State Univ., Columbus 10
- MAPP, GEORGE R., R. D. 1, Troy
- MARTIN, ROBERT L., 1936 Clinton St., Toledo
- MCCBRIDE, CHARLES GROVER, 2212 Glouster Rd., Columbus 12
- MCCCLURE, ROBERT J., 1957 Coventry Rd., Columbus 8
- MCGREW, C. D., Plumb Hall, Ohio State Univ., Columbus 10
- MEACHEM, WILLIAM S., 1509 E. Broad St., Columbus
- MEISTER, ARTHUR E., 650 Leonard St., Toledo
- MILLER, ROY, R. D. 2, Canton
- MINDLING, LUTHER, 4665 W. Fork Rd., Apt. C-3, Cincinnati 23
- MITTEN, HORACE L., JR., Dairy Technology Dept., Ohio State Univ., Columbus 10
- MONROE, C. F., Ohio Agr. Expt. Station, Wooster
- MOORE, C. M., Cowles Chemical Co., 7016 Euclid Ave., Cleveland 2
- NADELIN, EUGENE PAUL, 819 Quimby Ave., Wooster
- NASS, ED, Box 264, The Fountain, Canfield
- NISBET, 58 N. Washington Ave., Columbus 15
- OTTING, H. E., 2701 Minerva Lake Rd., Columbus 11
- PAXTON, JOHN, 29 E. Norwich Ave., Columbus
- PFEFFER, JOHN C., 4279 Hegner, Cincinnati 36
- POUNDEN, WILLIAM D., Dairy Dept., Ohio Agr. Expt. Station, Wooster
- PRATT, AVERY D., Dairy Industry Dept., Ohio Agr. Expt. Station, Wooster
- PRATT, H. E., 226 Denison Ave., Elyria
- PUTNAM, DEXTER N., Prod. Testing Dept., American Jersey Cattle Club, Columbus 15
- RAMSEY, R. F., 21853 Cromwell, Rocky River Branch, Cleveland
- RATHBUN, GORDON P., 111 E. Forest St., Clyde
- RHOADES, PAUL L., 2776 Albrecht Ave., Akron
- ROSS, RICHARD M., 8 E. Long St., Columbus
- SALISBURY, S. M., Dept. of Dairy Husbandry, Ohio State Univ. Columbus 10
- SCHELLENGER, KERN K., 514 Woodbury Ave., Columbus 4
- SCHILLING, R. L., R. D. 1, Farmersville
- SCHROEB, C. J., 137 N. 8th St., Upper Sandusky
- SEWELL, W. E., The Procter & Gamble Co., M.A. & R. Bldg., Ivorydale 17
- SHAFFER, REED, Box 132, Greenville
- SHELTON, ELBERT M., 4614 Prospect Ave., Cleveland 8
- SLATTER, WALTER L., Dept. of Dairy Technology, Ohio State Univ., Columbus 10
- SMITH, DUKE L., 1320 Linda St., Rocky River 16
- SMITH, JAMES T., Box 5587, Cleveland 1
- SPRAGUE, A. L., Jackson Center Creamery, Jackson Center
- STARBUCK, RAYMOND R., Plumb Hall, Ohio State Univ., Columbus 10
- STEINER, JOHN, Akron Pure Milk Co., Akron
- STEVENS, SUE CASSELL, 1033 Salem Ave., Dayton 6
- STIVER, HUGH F., R. D. 2, Lewisburg
- STUCKEMAN, HOWARD W., Box 164, Kinsman
- SUTERMEISTER, L. A., Borden's Finch Farms, 219 E. 5th St., Dayton
- SUTTON, T. S., Dept. of Agr. Chemistry, Townshend Hall, Ohio State Univ., Columbus 10
- SWINEHART, MICHAEL F., 160 N. Lisbon St., Carrollton
- THOMAS, ROBERT C., U. S. Public Health Service, Kroger Bldg., Cincinnati 2
- TITUS, R. W., Control Lab., Nestles Milk Products, Marysville
- TUCKER, HUBERT, Worthington
- TUCKER, WALTER S., 5015 Monroe St., Box 129, Station B, Toledo
- VAN BUREN, RALPH, 15 Boehler St., Tiffin
- VOELLER, EARL H., Plumb Hall, Ohio State Univ., Columbus 10
- VRAMAN, V. S., Defiance Milk Products Co., Defiance
- WAGNER, WILLIAM, Canal Fulton
- WAY, H. O., 2403 Prospect Ave., Cleveland 15
- WEHR, C. G., 725 East Ave., Hamilton
- WEISER, HARRY H., Dept. of Bact., Ohio State Univ., Columbus 10
- WICKHAM, J. C., 4227 W. 36th St., Cleveland
- WIEDEMER, ARTHUR, 201 Euclid Ave., Greenville
- WILSON, HOEACE K., 330 E. Paint St., Washington C.H.
- WISCHHUSEN, J. F., 15031 Shore Acres Dr., Cleveland 10
- WOESKENBERT, NORBERT H., 1623 Rose Pl., Cincinnati 29
- WOODYARD, W. O., Cudahy Packing Co., Washington C.H.

Student Affiliates:

- ABELL, CARL T., 154 E. Woodruff, Columbus
- ANTEL, RICHARD K., 2625 Euclid Hts. Blvd., Cleveland 6

- ARGEROS, JOHN, Room 106 River Rd. Dorms, Columbus
 BALDWIN, CHARLES S., 868 Dennison Ave., Columbus
 BAUMAN, WARREN, 3 14th Ave., Columbus
 BAXTER, RICHARD C., 333 W. 5th St., Delphos
 BEARDSLEY, JOHN E., 2208 Indiana Ave., Columbus
 BEERY, CARL, R. D. 1, Lancaster
 BENDER, ROLAND D., 87 W. Northwood, Columbus
 BENSON, KIRK, 123 Selby Blvd., Worthington
 BETZEL, FRED, 290 Garden Rd., Columbus
 BOTSCH, R. M., R.D. 2, Grafton
 BRICKER, DUANE, R. D. 3, Massillon
 BROWN, KENNETH, Cristdale Farm, Route 2, Loudonville
 BUNDUS, ROBERT, 2005 Summit St., Columbus
 BUSKENMEYER, DALE F., Route 3, Box 32, Swanton
 CARSON, HERMAN, 1957 Indianola Ave., Columbus
 CHAPMAN, GEORGE A., 60 E. Lane Ave., Columbus
 CLAY, FRED, 1478 N. 5th St., Columbus
 CLAYTON, KEITH, 1831 N. 4th St., Columbus
 COFFMAN, FRED E., R. D. 1, Germantown
 CONNELLY, ROBERT M., 27 Boulevard, Shelby
 CONRAD, HARRY R., Dairy Husbandry Dept., Ohio State Univ., Columbus 10
 COOPER, PAUL, 33 14th Ave., Columbus
 COPE, HOWARD D., 429 McKinley Ave., Crooksville
 CRECELIUS, BOB, Allen Hotel, Bellevue
 CULP, WILLIAM R., 606½ Monroe St., Martins Ferry
 CURTIS, VIRGIL, 287 E. Tulane Rd., Columbus
 DAVIS, CHARLES, 266 S. Knox Rd., Apt. D, Columbus
 DAVIS, HOWARD F., 23 Chittenden Ave., Columbus
 DEBROSSE, A. C., P. O. Box, University Station, Columbus
 DENTNER, RICHARD, 334 N. Burgess Ave., Columbus
 DIETRICH, JOHN P., 5019 N. High St., Columbus
 DUDLEY, DAVID, 1772 W. 1st Ave., Columbus
 DULING, WILLIAM, Route 4, Coshocton
 EBERT, EDWARD, R. D. 1, Lockbourne
 EBERT, WILLIAM, 740 Kimball Pl., Columbus
 ELTZROTH, EARL, 1957 Indianola Ave., Columbus
 EEZEL, MILES, 1373 E. Hudson St., Columbus
 FARISON, RICHARD B., Box 29, McClure
 FECHHEIMER, NATHAN, Hopewell Rd., Montgomery
 FISCHER, JESSIE, Baker Hall, Box 219, Ohio State Univ., Columbus 10
 FLANAGAN, PAUL, 141 13th Ave., Columbus
 FOGG, DONALD E., Court House, Lancaster
 FOSTER, DEAN, R. D. 3, London
 FOWLER, WILLIAM, 609 Spruce St., Caldwell
 FOX, CARMEN, R. F. D. 1, Seville
 FOX, KENNETH, 171 E. Northwood, Columbus
 FREEBIS, JAMES H., 341 W. 6th Ave., Columbus
 GARMAN, WALTER, 25 W. Frambes Ave., Columbus
 GEPHART, WILLIAM A., 1339 Ducksberry Ave., Columbus
 GIFFORD, MYRON, R. D. 1, Galena
 GLASS, DALE, 3377 Kenny Rd., Columbus
 GORDIE, GERALD H., 1918 Indianola Ave., Columbus
 GUIDER, DONALD, 64 W. Duncan St., Columbus
 HARPER, W. JAMES, Dairy Dept., Ohio State Univ., Columbus
 HARSTER, ROBERT, R. D. 1, Jeromesville
 HARTLEY, RICHARD, Dairy Technology Dept., Ohio State Univ., Columbus 10
 HAWK, GEORGE E., 611A Hess Rd., Columbus 10
 HAWTHORNE, MORRIS, 126 Chittenden Ave., Columbus
 HOFIUS, DONALD, Ohio Club, Stadium Dorm, Ohio State Univ., Columbus 1
 HOOVER, A. S., 461 E. Sandusky St., Findlay
 HOOVER, B. J., 2656 Tremont Rd., Columbus
 HOWE, DONALD, 2097 Summit St., Columbus
 JOHNSON, HENRY R., 550 W. Ontario, Lima
 JONES, DAVID, Route 1, Delaware
 JOYCE, JOHN B., JR., 332 Westland Ave., Columbus 9
 KAGY, DONALD, 1230 Neil Ave., Columbus
 KENSINGER, WILLIAM S., R. D. 2, Greenfield
 KISHMAN, GEORGE, 385 E. Tulane Rd., Columbus
 KOHL, N. D., 430 N. Clairmont Ave., Springfield
 KOHLMORGAN, ELMER, 1825 Atlantic St. N.E., Warren
 KOVAL, FRANK, 70 E. 18th Ave., Columbus
 LADRACK, EUGENE, 427 W. Elm St., Washington C.H.
 LAISHLEY, P. L., Martin Bldg., Crestline
 LANGE, DONALD, Box 169, Washington C.H.
 LOO, CHING CHEE, 1020 River Rd. Dorm, Columbus 10
 LOWER, GENE, 12 University Place, Columbus 1
 LUTZ, HARRY C., 805 N. Market St., Troy
 MASSIE, JOHN W., 175 E. 13th Ave., Columbus
 MCKELVEY, PAUL L., 4480 Lincoln Ave., Shadyside
 MILFORD, WILLIAM DEAN, 70 2nd St., Rittman
 MOORE, WILBUR V., Pleasant City
 MUGRAVE, DONALD K., Wharton
 NADAL, R. A., Central YMCA, Columbus
 NEEL, LAWRENCE, JR., 510 N. Main St., Orrville
 NEUHARDT, VERNON A., R. D. 2, Lewisville
 OBERSCHLAKE, DWIGHT, Hamersville
 OWEN, GENE, River Rd. Dorms, Columbus

PANIGOT, EUGENE, 180 Brady Circle, Steubenville
 PAULIN, MARVIN E., R. D. 2, New Paris
 PETERSON, RAYMOND D., 504 Gallia St., Apt. D, Columbus
 POPLIN, PARRY M., 86 W. Main St., Carrollton
 PURDY, CLYDE L., Killbuck
 RENNER, KENNETH R., Dairy Technology Dept., Ohio State Univ., Columbus 10
 REVELO, GEORGE J., 114 Harrison St., Middletown
 RHODES, JAMES A., JR., R. D. 2, Salem
 RICHMOND, ROBERT N., R. F. D., Huntsburg
 RICKARD, HERMAN, R. D. 3, Albany
 RIDDLE, PAUL, 8005 W. Broad St., Galloway
 RIENKE, EUGENE, 3421 Lyleburn Place, Cincinnati 20
 RINEHART, L. VAUGHN, R. D. 1, Galion
 ROBERTS, JAMES, 386 S. Terrace, Columbus
 ROBINSON, ROBERT F., 224 Mound St., Logan
 ROUSH, DALE K., Southeastern Ohio Agr. Expt. Farm, Carpenter
 ROUSH, HAROLD, 141 W. Northwood Ave., Columbus
 RYDER, WENDELL, 1918 Indianola Ave., Columbus
 SCHELL, LEONARD, R. D. 2, Apple Creek
 SCHERER, ROBERT D., Haverhill
 SCHILLINGER, JOHN, 2684 Neil Ave., Columbus
 SCHMIDT, ROBERT E., 4355 St. Lawrence Ave., Cincinnati
 SCHNEIDER, JOHN W., Box 232, Sugarcreek
 SCHROEDER, HAROLD, Plumb Hall, Ohio State Univ., Columbus 10
 SCOTT, GEORGE, 1131 Deacon St., Springfield 10
 SEIBERLING, DALE A., 107 E. Lane Ave., Columbus
 SEMONES, VERNON, 4316 Gallia St., New Boston
 SENADENOS, SAM, 312 Tibet Rd., Columbus
 SHIFFER MILLER, WILLIAM E., 806½ N. Main St., Dayton
 SICKAFOOSE, DONALD G., 81 W. Frambes, Columbus
 SIDDALL, ROBERT L., 560 Roslyn Ave., Akron
 SIMMONS, AUGUST T., R. D. 3, Cambridge

SIMMONS, LLOYD V., JR., 56 W. Frambes Ave., Columbus
 SMITH, ROSS, 3422 Oakway Drive, Toledo 9
 SMOOTS, EDWARD, 2382 Brentwood Rd., Columbus
 SMYTH, FRANK, 807 River Rd. Dorm, Ohio State Univ., Columbus 10
 SNIDER, JOHN, River Rd. Dorms, Columbus 10
 SNODDY, WALTER, Shreve
 SPRANG, RALPH, 583 Hess Rd., Apt. B, Columbus
 SQUIRES, HAROLD C., 76 W. Woodruff Ave., Columbus
 STAFFORD, J. R., R. D. 2, Tipp City
 STANTON, JIM, 2357 Linden Ave., Columbus
 STARR, MERLE, 1957 Indianola Ave., Columbus
 STRACHLER, RAYMOND D., 412 Knecht Drive, Dayton
 SUMMERS, DONALD, R. D. 2, Urbana
 THARP, VICTOR, Blue Crystal Farm, Hilliard
 TISHER, KENNETH, 2280 N. High St., Columbus
 TITUS, JAMES B., Rutland
 VOGELGESANG, CHARLES, 927 10th St. N. E., Massillon
 VOZABULE, BERNARD T., Box 90, Southern Rd., Everett
 WABY, JOHN C., 82 W. 3rd, Columbus
 WEBB, WILLIE JR., 2272 N. High St., Columbus
 WEBER, EDSSEL V., R. D. 2, Box 43, Lewisville
 WEBER, EDWARD, 416 Glenwood Ave., Cincinnati
 WELLS, DALE, 86 W. Norwich Ave., Columbus
 WESTRICK, FRANCIS C., Route 3, Defiance
 WEINERY, GEORGE B., 81 E. College St., Alliance
 WITHERSPOON, WILLIAM, 459 E. Dunedin Rd., Columbus
 WHITTAKER, ARNOLD, 4409 Westway, Toledo 12
 YODER, ORIE, 1359 Dennison Ave., Columbus
 ZIEGLER, DONALD E., Apt. F, 2594 Putman St., Columbus

OKLAHOMA

Members:

BEROUSEK, E. R., Dept. of Dairying, Okla. A & M, Stillwater
 BOEHR, JOHN W., Extension Service, Okla. A & M, Stillwater
 BURKEY, JOHN P., 121 S. Hugh, Cushing
 CAVE, H. W., Dept. of Dairy Husbandry, Okla. A & M, Stillwater
 COLVERT, RICHARD W., Ardmore
 CROWNOVER, WILLIAM M., Box 52, Pawhuska
 GATHERS, WAYNE E., Box 1536, Tulsa
 GIBSON, WENDELL, 1712 S. Yorktown, Tulsa 4
 HOLLAND, THEODIES H., Langston Univ., Langston
 JANDER, JOHN C., A & M College Campus, Okmulgee

JOHNSON, PAUL E., Dept. of Dairying, Okla. A & M College, Stillwater
 KUHLMAN, A. H., Dairy Dept., Okla. A & M, Stillwater
 LEWIS, J. C., 319 W. Pierce, Mangum
 LOEWENSTEIN, MORRISON, Dept. of Dairying, Okla. A & M, Stillwater
 MACVICAR, ROBERT, Agr. Chemistry Research, Okla. A & M, Stillwater
 MCGILLIARD, P. C., Dairy Dept., Okla. A & M, Stillwater
 OLSON, H. C., Dept. of Dairying Okla. A & M, Stillwater
 SCHWABE, LEE R., Carnation Co. of Okla., Tulsa
 STINNETT, L. H., Extension Service, Okla. A & M, Stillwater

THOMASON, LOUIS C., 713 N. Davis, Enid
 TRZCINSKI, SAM C., 2030 Denver, Muskogee

Student Affiliates:

ANDERSON, ROBERT E., 314 Knoblock, Stillwater
 BAKER, JAMES B., 102 Stadium, Okla. A & M, Stillwater
 BARBER, LEWIS F., JR., Box 85, Veterans Village, Stillwater
 BASSE, ROBERT W., 240 Duck St., Stillwater
 BURTON, CHARLES L., 2614 S. Shartel, Oklahoma City
 COFFIELD, A. W., Box 626, Drumright
 CORE, WILLIAM J., P.O. Box 815, Homin
 DAVIS, ALBERT O., 463 Cordell Hall, Okla. A & M, Stillwater
 DEEDS, ESMUEL W., Box 664, Veteran's Village, Stillwater
 EDMONDSON, LOCKE F., 1411 Hartford, Stillwater
 EISENHAEUER, CHARLES, R. R. 3, Newkirk
 FOSHEE, WILLIAM A., 402 Washington St., Stillwater
 GOODS, AL, Route 1, Poteau
 GUTHRIE, CHARLES K., 511 W. 7th, Stillwater
 GUYER, DAN E., 1204 S. W. 31st St., Oklahoma City
 HAMILTON, BILLY G., 264 Cordell Hall, Stillwater
 HANKINS, JAMES, 517½ West St., Stillwater
 HARDIN, GEORGE, Route 2, Stillwater
 HARRINGTON, DWAIN F., Box 643, Veteran's Village, Stillwater
 HAYS, JIM L., 1339 N. Atlanta
 HICKEY, BRYCE, JR., 418 S. Independence, Sapulpa
 KEIRN, DOYLE L., Box 655, Veteran's Village, Stillwater
 KEIRN, ORAN R., R. D. 3, Glencoe
 KERNAN, FRANK H., JR., Box 837, Veteran's Village, Stillwater
 KNIPE, DICK, No. 10 LaRue Midi, Veteran's Village, Stillwater
 KOZEL, KARL A., R. D. 3, Shawnee
 LAUBACH, MARVIN R., 201 West St., Stillwater
 LONG, JOHN EDWARD, 201 Duncan, Stillwater
 LONG, ORRIAN B., Hallett
 MCCARROLL, L. M., 2336 B. N. E., Miami
 McDOWELL, RALPH L., 240 Hester, Stillwater

MITCHELL, ESTES D., 323 West St., Stillwater
 PARKER, GEORGE M., Box 528, Veteran's Village, Stillwater
 PARKER, GEORGE M., Unit 10, Apt. 7, Jordan St., Stillwater
 PARSONS, G. F., JR., Box 731, Idabel
 PERKINS, BILLY GENE, Route 2, Wewoka
 PITTMAN, ARMON A., 7 E. Woodrow Place, Tulsa 6
 PORTER, PHILIP B., Box 78, Veteran's Village, Stillwater
 PRESCOTT, HURSTON E., 121 S. Main, Stillwater
 PROPHET, RUSSELL E., Box 873 Veteran's Village, Stillwater
 REECE, M. F., JR., Route 2, Stillwater
 RUPPERT, BOB J., 267 Cordell Hall, Stillwater
 SCHMIDT, KENNETH F., 210 N. G St., Muskogee
 SCHMIDT, SAMUEL, Box 526, Stillwater
 SHARKEY, ROY, JR., Pond Creek
 SHEETS, BOB, Route 1, Box 219, Tulsa
 SMITH, ARTHUR J., 267 Cordell Hall, Stillwater
 SMITH, JAMES D., 321 N. Duck, Stillwater
 SMITH, JOHN A., 619 Market St., Muskogee
 STACY, J. W., New Castle
 STAHL, PERRY, 408 Duck St., Stillwater
 STEPHENS, BOBBY JOE, 461 Cordell Hall, Okla. A & M, Stillwater
 STEPHENS, WILLIE O., 1523 W. 6th St., Stillwater
 STOUT, VIRGIL E., 401 S. Townsend, Ada
 STREICH, MERLE, 302 Knoblock, Stillwater
 TARRANT, HAROLD, 307 Hanner Hall, Stillwater
 THOMAS, WILLIAM, R., 713 College Ave., Stillwater
 VON GUNTEN, R. L., 1806 College Ave., Stillwater
 WALKER, CHARLES H., Dairy Dept., Okla. A & M, Stillwater
 WILLIAMS, GRADY F., Dairy Dept., Okla. A & M School of Tech. Training, Okmulgee
 WILLIAMS, RALPH, Box 554, Veteran's Village, Stillwater
 WILSON, ROBERT W., 707 Adams, Stillwater
 WILSON, PAUL E., 240 Husband, Apt. 1, Stillwater
 WOODS, WILLIAM H., 354 Cordell Hall, Okla. A & M, Stillwater

OREGON

Members:

BRANDT, LEWIS C., 28 N. 22nd St., Corvallis
 BRANDT, P. M., Div. of Animal Industries, Ore. State Agr. College, Corvallis
 BYERS, JOHN H., Dairy Dept., Ore. State College, Corvallis
 ELLIKER, PAUL R., Dept. of Bact., Ore. State College, Corvallis
 EWALT, H. P., 309 Dairy Bldg., Ore. State College, Corvallis

GEORGE, J. S., Tillamook Co. Creamery Ass'n., Tillamook
 GLYNN, J. K., 1935 S. W. Park, Portland
 HAAG, J. R., Agr. Expt. Station, Corvallis
 HOWELL, H. B., Route 1, Box 921, J. J. Astor Expt. Station, Astoria
 JONES, IDWAL R., Dairy Dept., Ore. State College, Corvallis
 KEYSER, H. C., c/o Dairy's Supply Co., Inc., 506 S. E. Union Ave., Portland

KLAUS, FRED C., 138 S. Liberty, Salem
 LONGWELL, BRYANT R., Consolidated Food
 Co., Cornelius
 MCKENZIE, FRED, Ore. State College, Cor-
 vallis
 MEADOWS, ELMER J., 302 Corbett Bldg.,
 Portland 4
 MILLER, DONALD, Dept. of Bact., Ore. State
 College, Corvallis
 MORSE, ROGER W., Extension Dairyman, Ore.
 College, Corvallis
 OLOUFA, MOHAMED M., 620 N. 21st St., Cor-
 vallis
 RICHARDSON, GEORGE A., Dept. of Dairy
 Husbandry, Ore. State College, Corvallis
 ROWE, GORDON A., Dairy Bldg., Room 307,
 Ore. State College, Corvallis
 STEIN, ROY W., 1313 S. E. 12th St., Portland
 SWANSON, EDGAR H., Dairy Coop. Ass'n.,
 1313 S. E. 12th Ave., Portland 14
 WAGGONER, RALPH W., Box 333, Klamath
 Falls
 WEBSTER, HARRY L., Clatskanie
 WILSTER, G. H., Dept. of Dairy Husbandry,
 Ore. State College, Corvallis

Student Affiliates:

BALSARA, DARIUS S., Dept. of Food Tech-
 nology, Ore. State College, Corvallis
 BEEG, LESLIE R., c/o Langlois Cheese
 Makers, Langlois

PENNSYLVANIA

Members:

ALMQUIST, JOHN, O., Dept. of Dairy Hus-
 bandry, Penn. State College, State College
 ANDERS, HERBERT K., Agr. Extension Ass'n.,
 Box 487, Williamsport
 ARNOLD, RICHARD N., R. D. 9, York
 AUSTIN, CARLTON J., Supplee-Wills-Jones
 Milk Co., 15 S. 34th St., Philadelphia 4
 BARNHART, JOHN L., Swede Rd., Norristown
 BEAM, A. LELAND, 141 Ridge Ave., State
 College
 BENNY, WALTER, 195 E. Main St., Bradford
 BORLAND, A. A., 310 S. Burrowes St., State
 College
 BORTREE, ALFRED L., Dept. of Animal Hus-
 bandry, Penn. State College, State College
 BEACKE, GEORGE M., Rieck-McJunkin Dairy
 Co., 1345 Forbes St., Pittsburgh
 BROCK, O. CARLYLE, 519-29 E. 18th St., Erie
 BRYAN, PAUL S., R. D. 1, Perkasio
 CLAXTON, A. J., Meadow Gold Dairies, Inc.,
 126 Denniston Ave., Pittsburgh
 COHRE, C. I., Philadelphia Inter-State Dairy
 Council, 234 S. 22nd St., Philadelphia 3
 COLDRÉN, JOHN, 700 W. Middle St., Hanover
 COLEMAN, PAUL E., R. D. 1, Wexford
 CONE, J. FRANK, Dept. of Bacteriology,
 Penn. State College, State College
 DAHLE, CHESTER D., Dairy Dept., State
 College
 DAVEY, WILLIAM R., Room 125, Dairy Bldg.,
 State College
 DAWDY, MAX L., Dairy Dept., Penn. State
 College, State College

BERGER, PAUL C., Route 3, Albany
 BURCH, I. M., Route 1, Box 273, Corvallis
 CORNETT, JOHN A., Jr., 32 N. 26th St.,
 Corvallis
 COVINGTON, J. L., 637 N. 14th, Corvallis
 COWGILL, FORREST L., 231 Mall St., Corvallis
 DALAL, SAM, 440 N. 6th St., Corvallis
 GANGER, R., 8 N. 27th, Corvallis
 GARNER, LYEEL B., Route 1, Box 323, Gaston
 JACOBS, BRUTAS L., Route 4, Box 181, Cor-
 vallis
 MURRAY, R. N., 812 Pacific Terrace, Klamath
 Falls
 OLSON, ALVIN E., 2040 Jackson St., Corvallis
 PARFIA, H. A. B., Oregon State College,
 Corvallis
 REGELE, RODGER L., 942 N. 9th St., Corvallis
 ROCK, WILFORD D., 2500 Monroe St., Cor-
 vallis
 SCHMIDLKOFER, JOSEPH C., 129 W. Douglas,
 St. Roseburg
 STREET, MARION C., 135 S. 9th St., Corvallis
 STEIFF, ROBERT, 6140 S. W. Shattuck Rd.,
 Portland 19
 WALLACE, ARTHUR J., 9844 N. E. Pacific St.,
 Portland
 YOUNG, KEITH F., 617 S. E. Main St., Port-
 land
 YOUNG, ORVILLE, 207 N. 21st St., Corvallis

DEUBLER, E. C., Bucks County, Newtown
 DOAN, F. J., Dairy Dept. Penn. State Col-
 lege, State College
 DRAGGO, EUGENE T., 35 W. Pine St.,
 Knightstown
 DUTCHER, R. ADAMS, 254 E. Hamilton Ave.,
 State College
 EDINGER, FLOYD G., 429 N. Main Ave.,
 Scranton 4
 ERCH, A. GLYNN, Lewisburg
 GALLICKER, LOUIS, Gallicker Ice Cream Co.,
 453 Franklin St., Johnstown
 GEARHART, C. R., Penn. State College, State
 College
 GEYER, FRANK E., 601 Laver Rock Rd.,
 Glenside
 GIFFORD, CLAUDE W., Farm Journal, Inc.,
 Washington Square, Philadelphia 5
 GILMORE, HERBERT C., 213 Dairy Bldg.,
 State College
 GOSSE, HAROLD, 409 Warrington Ave.,
 Pittsburgh 10
 GREENLEAF, FREDERICK M., 1724 Congress
 St., Allentown
 GROW, WALTER, Fluid Milk Plant, 265 Beech
 St., Pottstown
 GUERRANT, N. B., Penn. State College, State
 College
 HADFIELD, WILLIAM A., Penn. Salt Mfg.
 Co., 1000 Widener Bldg., Philadelphia 7
 HAMOR, WILLIAM A., Mellon Institute, 4400
 5th Ave., Pittsburgh 13
 HIDINGER, ORVIS, 204 Times Bldg., Penn.
 Ave. & Spruce St., Scranton

HORN, DAVID WILBUR, Box 666, Bryn Mawr
 JAGGARD, GEORGE, 2300 Locust St., Philadelphia 3
 JOHNSON, H. S., 119 Federal St., Pittsburgh 12
 JOHNSON, LAWRENCE W., Whitmoyer Laboratories, Inc., Myerstown
 JONES, P. D., Dairy Dept., Penn. State College, State College
 JOSEPHSON, D. V., Dairy Dept., Penn. State College, State College
 JUZI, WILLIAM H., Guernsey Breeders Assoc., Box 491, Harrisburg
 KEEN, ROBERT H., Queen Dairy Co., Inc., 413-19 S. Queen St., Lancaster
 KENT, ALBERT L., R. D. 1, New Albany
 KESLER, EARL M., Dairy Husbandry Dept., Penn. State College, State College
 KILGORE, JAMES, Route 1, Northampton
 KING, CLIFTON J., Box 25, Dayton Armstrong Co.
 KIRK, J. J., 409 Maple Ave., DuBois
 KNOTT, C. B., Dept. of Dairy Husbandry, Penn. State College, State College
 LAUGHLIN, JOHN, Menzie Dairy Co., 1909 River St., McKeesport
 LAWYER, ROBERT K., 397 E. Upsal St., Philadelphia 19
 McCANDLESS, J. COULTER, R. D. 5, Butler
 McCURE, JOSEPH W., New Wilmington
 McGRATH, GERALD, 3010 N. 3rd St., Philadelphia
 McMICHAEL, HARRY S., Meadow Gold Dairies, Inc., 126 Denniston Ave., Pittsburgh 6
 MEISENHOLDER, SAM F., R. D. 2, Dover
 MELIK, LA RUE T., 426 Memorial Ave., Grove City
 MERRILL, HOWARD J., 206 Dairy Bldg., State College
 MOORE, ROBERT B., 1808 W. 24th St., Erie
 NAGEOTTE, JOE, Dairy Extension Specialist, 213 Dairy Bldg., State College
 OLMSTEAD, R. H., Dairy Bldg. Penn. State College, State College
 OTTO, B. FRANK, Otto Suburban Dairy, Inc., Camp Horne Rd., Box 4198 Bellevue, Pittsburgh
 PARKHURST, RAYMOND T., Flory Milling Co., Bangor
 PARKIN, IVAN E., Room 213, Dairy Extension, State College
 PATTON, STUART, Dairy Husbandry Dept., Penn. State College, State College
 PECORA, PASQUO P., Pecoradale Farms, R. D., Drums
 PERRY, N. A., Dairy Bldg., Penn. State College, State College
 PRATT, HAROLD T., Philadelphia Dairy Prod. Co., Box 267, Philadelphia 5
 PRINCE, PAUL W., Dept. of Dairy Husbandry, Penn. State College, State College
 RAHAUSER, GEORGE W., 5028 Smithfield Rd., Drexel
 RIETH, HOWARD E., R. D. 1, Box 96, Perkasie
 ROBERTSON E. I., John W. Eshelman & Sons, Lancaster

ROBINSON, CLYDE S., Coal Center
 ROLAND, CHARLES T., P.O. Box 1346, Calgon, Inc., Pittsburgh
 ROSENBAUM, ROBERT, 3743 D St., Philadelphia 24
 ROUGHT, GEORGE B., Nicholson
 SALLER, MORTON, Water Callowhill, Philadelphia 23
 SAMPEY, J. J., Abbotts Dairies, Inc., 238 Lombard St., Philadelphia 47
 SCHAEFER, WILLIAM F., JR., 48 Wyoming Ave., Tundhannock
 SCHWENK, JOHN P., R. F. D. 1, Allentown
 SCOTT, JOSEPH C., 52 S. Vireo Drive, McKees Rocks
 SHAFFER, HARVEY E., Dept. of Dairy Husbandry, Penn. State College, State College
 SMITH, ROBERT T., 311 Chamber of Commerce, Scranton
 SPUR, BERNHARD, 4317 Spruce St., Philadelphia 4
 SWOPE, W. D., Penn. State College, State College
 TANABE, T., Dept. of Dairy Husbandry, Penn. State College, State College
 TAYLOR, JOE S., Dairy Bldg., Penn. State College, State College
 THOMPSON, GEORGE W., Box 303, Clarion
 WALKER, H. S., 623 15th Ave., Bethlehem
 WATROUS, G. H., JR., Dept. of Dairy Husbandry, Penn. State College, State College
 WELCH, R. C., c/o Wilbur-Suchard Chocolate Co., Lititz
 WILLIAMS, PAUL S., Dairy Bldg., State College
 WOODRUFF, ALFRED C., 42 Locust Ave., Springfield, Delaware Co.
 YODER, DAVID, Landisville
 ZIMMERMAN, JOSEPH A., Lehighton

Student Affiliates:

COLLINS, WILLIAM F., Dairy Dept., State College
 ELDER, CHARLES, 2330 Woodstock Ave., Swissdale 18
 GANT, JOHN W., R. D. 1, Scottdale
 GROBMAN, SYDNEY, 2625 S. 9th St., Philadelphia 48
 HOKE, DRUSILLA, 80 N. Harrison Ave., Bellevue, Pittsburgh 2
 KEENEY, DAVID G., Dept. of Dairy Husbandry, Penn. State College, State College
 KEENEY, MARK, Dairy Husbandry Dept., Penn. State College, State College
 MUSSETT, ARTHUR T., Dairy Husbandry Dept., Penn. State College, State College
 PAINTER, HERVEY M., Route 1, State College
 SHOPE, FRANK L., 236 E. Foster Ave., State College
 SMITH, ARNOLD C., Dept. of Dairy Husbandry, Penn. State College, State College
 STINE, CHARLES M., 507 Curtin St., Osceola Mills
 WILDASIN, HARRY F., 127 S. Barnard St., State College
 WILLIAMS, JESSE B., Room 206, Dept. of Dairy Husbandry, Penn. State College, State College

PERU

Student Affiliates:

CAPURRO, JUAN MIGUEL, Casilla 468, Lima

PORTO RICO

Members:

BREÑES, LUIS RIVERA, Agr. Expt. Station,
Rio Piedros

MARTORELL, MIGUEL A., Box 28, Dorado

RHODE ISLAND

Members:

ARMSTONG, ROBERT E., 102 Summitt St.,
East Providence
ATWOOD, JOHN W., R. I. State College,
Kingston
CAMPBELL, MASON, Kingston

HENDERSON, B. W., JR., Fort Kearny, Saun-
derstown
NORTON, CHARLES L., R. I. State College,
Kingston
SMITH, WILLIAM T., 172 Berkshire St.,
Providence

SOUTH AMERICA

Student Affiliates:

BURGUEIRA D, GERMAN G., c/o Merido Mo-
tors, Merida Edo Merida, Venezuela

RUEDA, ING LEOPOLDO, Compania Colombi-
ana De, Alimentos Lacteos S. A., Bugala-
grande Valle, Colombia

SOUTH CAROLINA

Members:

BOWERS, T. S., Belton
BRANDT, GRAYDON W., Dairy Dept., Clemson
Agr. College, Clemson
BRANNON, CARROLL C., Dairy Dept., Clem-
son College, Clemson
BREAHALE, THOMAS C., JR., Dairy Dept.,
Clemson College, Clemson
COBB, E. D., Pet Dairy Products Co., Green-
ville
FLETCHER, O. J., McColl
GEE, GASTON, State Farm, Boykin
GOODALE, B. E., Clemson College, Clemson
HELLAMS, J. I., Greenwood Creamery Co.,
Greenwood
HIERS, JESSE C., c/o County Agent, Ander-
son
HOBSON, W. M., Foremost Dairies, Spartan-
burg
HURST, VICTOR, Dairy Dept., S. Carolina
Agr. Expt. Station, Clemson

KAPP, ROBERT P., Edisto Farms Dairy, Co-
lumbia
KELLY, J. W., 12 Bishop St., Inman
KING, WILLIS A., Dairy Dept., Clemson Col-
lege, Clemson
LA MASTER, J. P., Dairy Dept., Clemson
College, Clemson
LAZAR, JAMES T., Dairy Dept., Clemson
College, Clemson
LOMAS, C. H., Clemson College, Clemson
LYLE, JOHN W., JR., 21 Forest Lane, Clem-
son
MERCER, DONALD N., R. F. D., Lykesland
WHISENHUNT, HENRY L., Mimosa Farm,
Cope

Student Affiliates:

GILPIN, A. T., Box 762, Clemson
HANCKEL, RICHARDSON M., Coburg Dairy,
Charleston
PETTIGREW, J. E., Route 2, Box 1, Ander-
son

SOUTH DAKOTA

Members:

BARTLE, EMERY, Box 3, College Station,
Brookings
BREAHALE, D. F., Dairy Husbandry Dept.,
S. Dak. State College, Brookings
CAVE, R. A., S. Dak. State College, Brook-
ings
DRACY, ARTHUR E., Dairy Husbandry Dept.,
S. Dak. State College, Brookings
EDWARDS, WILLIAM CHARLES, Tyndall
GOODBARY W. ALLAN, Dairy Dept., S. Dak.
State College, Brookings
HAINES, A. L., 631 St. Charles, Rapid City

JOHNSON, LESLIE E., Animal Husbandry
Dept., College Station, Brookings
KELLY, P. L., Dairy Dept., State College
Station, Brookings
TOTMAN, C. C., Dairy Dept., S. Dak. State
College, Brookings
TURNER, GEORGE E., Dairy Dept., S. Dak.
State College, Brookings

Student Affiliates:

DEHOOGH, MARVIN, Route 1, Marion
PETERSON, REUBEN, Dairy Dept., State Col-
lege, Brookings

TENNESSEE

Members:

ARMSTRONG, W. H., 4222 S. Garden Rd.,
Knoxville

BENNETT, BALLARD K., W. Main St., Le-
gannon
CHAFFELL, CLYDE, 212 Pythian Bldg., Jack-
son

COLEBANK, L. O., Agr. Extension Service,
Univ. of Tenn., Knoxville
COPELAND, LYNN, Univ. of Tenn., Agr. Ex-
tension Office, 2321 West End Ave., Nash-
ville
DOUGHERTY, A. F., Southern Dairies, 601
Oak St., Knoxville
FRIEND, L. F., 1497 Union Ave., Unit 4,
Memphis
GARRECHT, HUBERT, Klinke Bros. Dairy,
2469 Summer Ave., Memphis
HARRISON, THOMAS B., 2307 Laurel Ave.,
Knoxville
HURLEY, W. C., A & I College, Nashville
HUTSON, C. A., Agr. Expt. Service, P.O.
Box 1071, Knoxville
LUSH, B. H., Dept. of Dairying, Univ. of
Tenn., Knoxville 16
MADDUX, JAMES N., Lewisburg Dairy Expt.
Station, Lewisburg
MOSS, W. F., #4 Federal Bldg., Chatta-
nooga
NAIVE, J. B., Beverly Hills Sanatorium,
Knoxville
OVERCAST, WOODROW W., Dairy Industry
Dept., Univ. of Tenn., Knoxville
PARSONS, V. D., 205 N. Ault St., Knoxville

RECORD, P. R., c/o Security Mills, Knoxville
ROGERS, L. R., Security Mills, Knoxville
RUBIS, JOSEPH A., 608 Shady Lane, Nash-
ville
SWANSON, ERIC W., Dairy Dept., Univ. of
Tenn., Knoxville
WYLIE, C. E., Dairy Dept., Univ. of Tenn.,
Knoxville

Student Affiliates:

ALBRECHT, T. W., Dept. of Dairying, Univ.
of Tenn., Knoxville
CARRELL, EVERETT E., 1412 Highland Ave.,
Knoxville
DUNAGAN, HORACE H., Univ. of Tenn.,
Dairy Dept., Knoxville
HUMPHREYS, ROBERT H., Collierville
JORDAN, FRED L., 607 10th Ave. N., Nash-
ville
MCADAMS, J. B., R. D. 3, Henderson
MOORE, CARL, Route 2, Dayton
NICHOLS, JAMES R., Route 7, Jackson
SANDERS, HOYT, Dept. of Public Welfare,
309 Market St., Knoxville
SMITHSON, S. F., Route 2, Bradyville
WARREN, LLOYD DUFF, 100 S. 16th St.,
Nashville

TEXAS

Members:

CARDWELL, JOE T., Dairy Mfg. Dept., Texas
Tech. College, Lubbock
CHAPMAN, WILLIAM E., Box 564, Wichita
Falls
DEWEY, LLOYD G., Phenix Dairy, 1220
Smith St., Houston 2
EISENSTEIN, PHILIP P., 2534 South Blvd.,
Dallas
ENGRAM, L. W., Dairy Dept., Prairie View
A & M College, Prairie View
FEATRO, JOSEPH G., Dept. of Dairy Hus-
bandry, A & M College of Texas, College
Station
FROEDGE, J. RALPH, Fort Worth Poultry &
Egg Co., Inc., P.O. Box 1537, Fort Worth
GIBSON, G. G., Extension Dairyman, c/o Ex-
tension Service, College Station
HARMON, LAURENCE G., Dept. of Dairy
Manufactures, Texas Tech. College, Lub-
bock
HEEP, HERMAN F., Heep Jersey Farms,
Buda
HOECKER, WESLEY H., Dept. of Dairying,
Texas A & M College, College Station
HOMEYER, W. C., Arrow Mills, Inc., P.O.
Box 3012, Houston 1
HOWE, J. W., Division of Agr., Texas Col-
lege of Arts & Indus., Kingsville
JOHNSTON, J. B., 4236 Lovell Ave., Fort
Worth 7
JONES, CHARLES L., Box 563, Friona
KILGORE, J. P., c/o Eastland Creamery,
Eastland
LEIGHTON, R. E., Dairy Dept., Texas A &
M College, College Station
LIVELY, JACK, Route 5, Bluff View Farm,
Dallas

MADDEN, FRED W., 423 E. Main St., Grand
Prairie
METZGER, JACOB, P.O. Box 899, Dallas 1
MOORE, A. V., Dairy Dept., Texas A & M,
College Station
PEDERSON, M. G., c/o Price's Creameries,
Box 1572, El Paso
PRICE, ROBERT B., Price's Creameries, Inc.,
P.O. Box 1572, El Paso
REED, W. W., John Tarleton College, Ste-
phenville
ROBB, NOEL N., Production Mgr., Carna-
tion Co., Box 914, Houston 1
RUPEL, I. W., Dairy Husbandry Dept.,
Texas A & M College, College Station
SHEPARDSON, C. N., Dean of Agr., Texas
A & M College, College Station
TINNEY, WILLIAM H., 3312 25th St., Lub-
bock
TULLEY, W. C., Box 1688, Ft. Worth
WILLINGHAM, J. J., Dept. of Dairy Manu-
factures, Texas Tech. College, Lubbock
YENTZEN, VURELL A., Nederland

Student Affiliates:

BAKER, W. D., Dairy Dept., Texas Tech.
College, Lubbock
BECKER, GLENN, Box 643, Paducah
BENNETT, JACK, Dairy Dept., Texas Tech.
College, Lubbock
BLANEK, LOUIS J., Dairy Dept., Texas Tech.
College, Lubbock
CARMICHAEL, JACK C., Dairy Dept., Texas
Tech. College, Lubbock
CARVER, JAMES A., Dairy Dept., Texas Tech.
College, Lubbock
CAUDLE, JEHU, Dairy Dept., Texas Tech.
College, Lubbock

DAETER, CLARENCE L., JR., Dairy Dept., Texas Tech. College, Lubbock
 DENISON, C. W., 314 E. Cardwell, Brownfield
 FANN, WILLIAM, 3800 31st St., Port Arthur
 FRY, TRAVIS, Dairy Dept., Texas Tech. College, Lubbock
 GATHEE, FOY, Dept. of Dairy Mfg., Texas Tech. College, Lubbock
 GARDNER, SAMUEL DEAN, Dairy Dept., Texas Tech. College, Lubbock
 HALL, J. D., Dairy Dept., Texas Tech. College, Lubbock
 HOGAN, DONALD, Dept. of Dairy Mfg., Texas Tech. College, Lubbock
 JACKSON, ROYCE T., Dairy Dept., Texas Tech. College, Lubbock
 JOHNSON, SIDNEY A., Dairy Dept., Texas Tech. College, Lubbock
 MAXEY, CHESTER, Dept. of Dairy Mfg., Texas Tech. College, Lubbock
 PEEPLES, MILTON, Dept. of Dairy Mfg., Texas Tech. College, Lubbock
 PLUMMER, WILLIAM H., Dept. of Dairy Mfg., Texas Tech. College, Lubbock

RATCLIFF, LANTIS, Box 5123, College Station
 REEVES, J. E., Dairy Dept., Texas Tech. College, Lubbock
 SAWYER, HAROLD, Dairy Dept., Texas Tech. College, Lubbock
 SHOPE, RICHARD E., Box 661, Mission
 SMITH, HARVEY T., Dairy Dept., Texas Tech. College, Lubbock
 SMITH, LOTT B., Dept. of Dairy Mfg., Texas Tech. College, Lubbock
 SMITH, TERRY, Dairy Dept., Texas Tech. College, Lubbock
 SPROULS, HENRY, Dept. of Dairy Mfg., Texas Tech. College, Lubbock
 TOMLINSON, HORACE, Dept. of Dairy Mfg., Texas Tech. College, Lubbock
 VICKERS, DUNWARD, Dairy Dept., Texas Tech. College, Lubbock
 WEEMS, CLOY H., Dairy Dept., Texas Tech. College, Lubbock
 WILLIAMS, CHARLES A., Route 2, Memphis
 WILLIAMS, GRADY F., 1330 Graham Drive, Bryan

UTAH

Members:

ANDERSON, H. D., Ephraim
 BATEMAN, GEORGE Q., Utah State Agr. College, Logan
 CAINE, GEORGE B., Utah Agr. College, Logan
 CURTIS, L. R., 2184 Oneida St., Salt Lake City 5
 FERRIS, REED, c/o Nelson Ricks Creamery Co., 314 W. 3rd S., Salt Lake City 1
 HARDELL, ROBERT, 657 E. Center St., Logan
 HOSKISSON, WILLIAM A., 952 Windsor St., Salt Lake City 4
 LAMB, D. O., Box 2490, Salt Lake City 14
 LARSEN, PAUL B., Dept. of Dairy Husbandry, Utah State Agr. College, Logan
 MORRIS, A. J., Dairy Husbandry Dept., Utah State Agr. College, Logan
 QUAYLE, JOSEPH R., 375 N. 1st E., Logan
 RICH, LYMAN A., U.S.A.C. Extension Service, Logan

Student Affiliates:

ABBOT, MYRON S., 479 N. 5th E., Logan
 BENNION, STERLING, 711 E. 9th N., Logan

BEUTLER, R. F. D. 1, Smithfield
 BOSWELL, EVAN C., Woodruff Hall, Logan
 BROWN, EARL S., 2885 S. 3rd E., Apt. 3, Salt Lake City
 BUCK, RULON W., Prefab 1709, College Housing, Logan
 BURCH, GENE H., U.S.A.C. Creamery, Logan
 FITZGERALD, JACK F., Camus
 FORD, LYLE S., Wallsburg
 HAGEMAN, ALGER E., 416 N. 5th E., Skanchny Apts., Logan
 HENINGER, HAROLD E., Mendon
 HOGAN, MAX, Midvale
 KILPACK, G. J., R. F. D. 1, Logan
 KRAUTH, WALTER, Woodruff Hall, Logan
 MERRILL, GEORGE, Box 208, R. D. 1, River-ton
 MILLER, IVAN, College Housing #505, Logan
 PETERSON, SCOTT R., Peterson Bros., Holstein Farms, Box 922, Park City
 PRICE, SETH, Paradise
 SANDERS, DOAN C., JR., P.O. Box 342, Farmington

VERMONT

Members:

BLANDING, L. W. R., Holstein-Friesian Ass'n., Brattleboro
 BRADFIELD, ALEX, 210 Loomis St., Burlington
 BREMER, HARRY E., Dept. of Agr., State House, Montpelier
 CAMBURN, O. M., Vt. Agr. Expt. Station, 489 Main St., Burlington
 CHAFFER, L. L., Morrisville
 CONKLIN, C. T., Ayrshire Breeders Assoc., Brandon

ELLENBERGER, H. B., Univ. of Vt., Burlington
 FRAYER, JAMES M., Vt. Agr. Expt. Station, 489 Main St., Burlington
 HAMLEN, C. L., St. Albans
 NEWCITY, GLADWYN, 16 Henry St., Bellow Falls
 NEWLANDER, J. A., Univ. of Vt., Burlington
 NORTON, H. W., Holstein-Friesian Ass'n., Brattleboro
 RIDDELL, W. H., University of Vt., Burlington

STILES, ALBERT H., 32 Proctor Ave., S. Burlington
 WARNER, WILLIAM K., 96 Colchester Ave., Burlington

Student Affiliates:

RESI, LOUIS A., 236 South Prospeck St., Burlington

VIRGINIA

Members:

AULL, W. F., JR., Assoc. Dairy Husbandry, Extension Dept., Va. Poly. Institute, Blacksburg
 BUSSEY, J. C., Southern Dairies, Rocky Mt. Connelly, R. G., Dept of Dairying, College of Agr., Blacksburg
 ESPE, DWIGHT L., 1047 N. Edgewood St., Arlington
 FLORA, CARROLL C., Dairy Bldg., Va. Poly. Institute, Blacksburg
 FOSTER, ARDEN S., Assoc. Dairy Extension-man, Va. Poly. Institute, Blacksburg
 GREENBANK, GEORGE R., 632 24th St. S., Arlington
 HERRMANN, LOUIS F., 3022 N. Westmoreland Dr., Falls Church
 HOLDAWAY, C. W., Dept. of Dairy Husbandry, Va. Poly. Institute, Blacksburg
 KENNEDY, G. S., Health Dept., City of Roanoke, Roanoke 11
 MALLOY, T. E., Bland
 MILLER, W. C., Garst Bros. Dairy, Roanoke
 OMMOTT, B. J., 2702 Valley Dr., Alexandria
 OWEN, JOHN W., State Milk Commission, 1203 E. Broad St., Richmond 19
 PAIS, ALEXANDER A., Box 191, Pocahantas
 POMEROY, C. J., JR., Cedar Spring Farm, Quinton
 REAVES, P. M., Va. Poly. Institute, Blacksburg
 RENNIL, FRANK F., JR., 1810-12 W. Main St., Richmond
 SMITH, S. S., 1003 State Office Bldg., Richmond
 STROBEL, DAVID R., 518 Bellevue Dr., Apt. 11, Falls Church
 THOMPSON, NORMAN R., Dairy Husbandry Dept., Va. Poly. Institute, Blacksburg

TRELOGAN, HARRY C., 6025 N. 18th St., Arlington
 WEIRETHER, FRANCIS J., Merck & Co., Inc., Elkton
 WRIGHT, DEAN A., Ninovan Farms, Gordonville

Student Affiliates:

BARLOW, JOSEPH A., Box 5172, Va. Tech. Station, Blacksburg
 BROOKS, JAMES P., Gen. Delivery, Waynesboro
 CONRY, JOE E., JR., Gen. Delivery, Blacksburg
 ENGBERSON, RAY D., Box 6253, Va. Tech. Station, Blacksburg
 GILBERT, R. W., Box 5380, Va. Tech. Station, Blacksburg
 GROVE, FRANK T., Box 5371, Va. Tech. Station, Blacksburg
 HAMMOND, LEE P., Box 3903, Va. Tech. Station, Blacksburg
 ISBELL, EDWARD H., 1015 W. 42nd St., Richmond 24
 KEITH, COOK MASON, Route 1, Box 251, Roanoke
 KLINE, E. HARLEY, Box 4209, Va. Tech. Station, Blacksburg
 NEWLANDER, HAROLD A., Oceanair Apts., 725 Chester St., Norfolk 3
 NESTER, M. K., Box A-591, Va. Tech. Station, Blacksburg
 SINK, JAMES W., R. F. D. 1, Rocky Mount
 TRENT, CHARLES F., 106 W. Main St., Christiansburg
 WAGGONER, BEN, 1124 E. Ocean View Ave., Norfolk

WASHINGTON

Members:

ADAMS, CAMERON S., Division of Dairy & Livestock, State Dept. of Agr., Olympia
 ANDREWS, GEORGE, 10227 15 N. E., Seattle 55
 ASHWORTH, U. S., State College of Wash., Pullman
 BECK, A. J., 618 Whitman, Walla Walla
 BENDIXEN, H. A., Dairy Husbandry Dept., Wash. State College, Pullman
 BERRY, M. H., Carnation Milk Farms, Carnation
 BLOSSER, T. H., Dept. of Dairy Husbandry, Wash. State College, Pullman
 BONEY, M. M., Box 157, Bellingham
 BOWMAN, RALPH, 1727 N. Ledgerwood, Spokane 13
 COWAN, ROBERT, 635 Elliott Ave. W., Seattle 99

DAHLBERG, A. O., 2500 W. Viewmont Way, Seattle 99
 ELLINGTON, E. V., Dairy Dept., Wash. State College, Pullman
 ERB, R. E., Dept. of Dairy Husbandry, Wash. State College, Pullman
 FENTON, F. E., Inland Empire Dairy Assoc., 1803 W. 3rd Ave., Spokane
 FORDHAM, WAYNE W., 4628 Fowler Court, Apt. 55, Everett
 GAISER, J. L., Yakima Dairymens Assoc., 10 N. 5th Ave., Yakima
 GIBERSON, EBERT, 915 S. 3rd St., Apt. A, Tacoma 3
 GOHEEN, ROBERT L., 1312 King St., Bellingham
 GOLDING, N. S., Dairy Husbandry Dept., Wash. State College, Pullman

HILL, OTTO J., Washington Coop. Egg & Poultry Ass'n., 201 Elliott Ave. W., Seattle 99
 JANZEN, HAROLD W., Box 391, Vancouver
 KNOTT, J. C., Dairy Dept., Wash. State College, Pullman
 LARSON, HAROLD, 3823 S. Wilkeson St., Tacoma 8
 MANUS, LOUIS J., 328 Moscow Rd., Pullman
 MURDOCK, FENCI R., Asst. Dairy Husbandman, Western Wash. Expt. Station, Puyallup
 NICHOLS, M. B., Agr. Extension Service, Wash. State College, Pullman
 OLDENBURG, W. J., 1239 Rainier Ave., Seattle 44
 PROUTY, C. C., Wash. Agr. Expt. Station, State College of Wash., Pullman
 RUSSELL, JAMES, KITSAP Dairymens Ass'n., P.O. Box 314, Bremerton
 SHAW, A. O., Dept. of Dairy Husbandry, Wash. State College, Pullman
 SORENSEN, ERNEST M., Box 389, Chehalis
 SWEETING, BERT, Medosweet Dairies, Tacoma
 WALDO, D. R., Mt. Vernon

Student Affiliates:

ALLISON, ROBERT W., 705 Campus Ave., Pullman
 BENSHOOF, S. R., Apt. 39-C, S. Fairway, Pullman
 BLINDHEIM, ALVIN, 3231 S. Broadway, Everett

CHRISTENSEN, LAWRENCE, Dept. of Dairy Husbandry, Wash. State College, Pullman
 DELANEY, ERNEST N., Route 2, Box 142 A, Clarkston
 EHRLER, MELVIN H., Dept. of Dairy Husbandry, Wash. State College, Pullman
 EMIGH, JOHN A., 608 California, Pullman
 ERICHSEN, INGER, Dept. of Dairy Husbandry, Wash. State College, Pullman
 GRIMM, JOHN A., 310 Cedar St., Snohomish
 HALE, LEONARD G., 28A S. Fairway, Pullman
 HIBBS, R. A., Box 758, College Station, Pullman
 HOPE, EARL B., Dept. of Dairy Husbandry, Pullman
 HORTON, ARCHIE T., 206 W. Harrison, Pullman
 LARSON, JAMES E., South Prairie
 MCBRIDE, CARMAN A., Dept. of Dairy Husbandry, Wash. State College, Pullman
 MORRISON, ROGER A., 322 Sunset Drive, Pullman
 MURPHY, WILLIAM S., Box 193, College Station, Pullman
 ORR, OLIVER O., 610 Linden St., Pullman
 SNELL, THOMAS I., Route 2, Ellensburg
 VINTON, RICHARD L., 39-B South Fairway, Pullman
 WEGE, WILLIAM E., Stimson Hall, Wash. State College, Pullman
 WHITAKER, ROBERT P., Box 111, College Station, Pullman

WEST VIRGINIA

Members:

ACKERMAN, R. A., Univ. of W. Va., Dairy Dept., Morgantown
 BOWMAN, A. W., Coble Dairy Products, Martinsburg
 BRUYNEEL, A. L., 620 Gaston Ave., Fairmont
 FIKE, JAMES E., Dairy Dept., W. Va. Univ., Morgantown
 HEEBINK, GERALD, Extension Dairyman, Oglebay Hall, Morgantown
 HENDERSEN, H. O., College of Agr., Morgantown
 HUTCHINSON, HUGH F., Box 351, Lewisburg
 HYATT, GEORGE JR., Dairy Dept., W. Va. Univ., Morgantown
 PINSKY, HARRY, 57 17th St., Wheeling

PORTER, CLYDE C., Lesage
 REEVES, CHARLES V., 54 Monroe St., Elm Grove, Wheeling
 VAN LANDINGHAM, A. H., Agr. Expt. Station Morgantown
 WEESE, SAMUEL J., 227 Hagans Ave., Morgantown

Student Affiliates:

JOHNSTON, WILLIAM P., 150 Newton St., College Park, Morgantown
 KEATON, WOODSON B., 206 Grant Ave., Morgantown
 MITCHELL, WILLARD, Arden
 TYLER, WILBUR J., Dept. of Dairy Husbandry, W. Va. Univ., Morgantown

WISCONSIN

Members:

ALLEN, NAT N., Dept. of Dairy Husbandry, Univ. of Wis., Madison 6
 ALTON, ALVIN J., Taylor Freezer, Beloit
 ANDERSON, OLIN, Badger Breeders Coop., Shawano
 BARRETT, GEORGE R., R. R. 1, Middleton
 BAYLEY, NED D., Dept. of Dairy Husbandry, Univ. of Wis.
 BERNDT, FRITZ R., Box 214, Jackson
 BOHSTEDT, GUS, College of Agr., Univ. of Wis., Madison

BRACE, CHARLES C., Lone Rock
 BROWN, L. W., Dairy & Food Control Lab., Univ. of Wis., Madison
 BUSH, M. G., Box 28, Green Bay
 CALBERT, H. E., Dairy Industry Dept., Univ. of Wis., Madison
 CALL, ARA O., Western Condensing Co., Appleton
 CARLSON, JOHN, 515 N. Clark St., Appleton
 CHIBLEN, EARL S., Borden Co., Milwaukee
 CRAMER, A. J., 1642 Monroe St., Madison 5

- DREHER, W. H., Badger Breeders Coop., Shawano
- DUGDALE, B. R., 4150 Hiawatha Drive, Madison
- ELLERTON, MELVIN E., 2119 E. Woodstock Place, Milwaukee 2
- ERIKSON, ARTHUR B., Lakeshire-Marty Co., Plymouth
- FABRICIUS, N. E., Ladysmith Milk Prod. Coop. Ass'n., Box 167, Ladysmith
- FARNHAM, MERLE G., Box 192, E. R. 14, Milwaukee 14
- FEUTZ, FRED, Lakeshire-Marty Co., Monroe
- FORSTER, T. L., Dept. of Dairy Industry, Univ. of Wis., Madison
- FOSTER, E. M., Agr. Hall, Univ. of Wis., Madison
- FRAZIER, WILLIAM C., College of Agr., Univ. of Wis., Madison
- GOULD, GEORGE B., Outagamie Producers Coop., Black Creek
- GREEN, THOMAS R., Kewaskum Dairy, Kewaskum
- HALADA, JEROME A., 220 S. 2nd St., River Falls
- HALES, M. W., Chr. Hansen's Lab., Inc., 9015 W. Maple St., Milwaukee 14
- HALL, SUMNAR A., P. O. Box 859, Station A, Green Bay
- HANSEN, GLEN, 1252 S. Forwell, Eau Claire
- HARDING, H. A., Baileys Harbor
- HARRIS, ROY T., Dairy Records Office, 207 King Hall, Madison
- HART, E. B., Dept. of Biochemistry, Univ. of Wis., Madison
- HARTMAN, G. H., White House Milk Co., 102 Revere Dr., Manitowoc
- HASKELL, W. H., Klenszade Prod., Inc., Beloit
- HEIZER, EDWIN E., Dairy Husbandry Dept., Univ. of Wis., Madison
- HENDRICKSON, F. A., Dairy Husbandry Dept., College of Agr., Madison
- HOILE, EDMUND G., 931 W. Elsie St., Appleton
- JACKSON, H. C., Dairy Dept., Univ. of Wis., Madison
- JANZEN, J. J., Dept. of Dairy Industry, Univ. of Wis., Madison
- JOHNSON, S. H., The Borden Co., Waukesha
- JOHNSON, BRUCE C., Dept. of Dairy Husbandry, Univ. of Wis., Madison
- JOHNSON, C. R., The Baker Laboratories, Inc., East Troy
- JORDAN, W. G., The Borden Co., Waukesha
- KASLER, GALE R., 620 N. Eighth St., Milwaukee 3
- KASSUBE, DUANE A., 1359 W. Wisconsin Ave., Appleton
- KLUETER, HARRY, Room 704, 1 W. Main St., Madison
- KNOX, WILLIAM D., c/o Hoard's Dairyman, Fort Atkinson
- KOCH, HARLAN R., 4346 Hillcrest Dr., Madison
- KRAUSE, F. W., Meier Ice Cream Co., Waukesha
- LEGRIID, LESTER I., Dairy Division, Wis. State Dept. of Agri., State Capital, Madison
- MASTERS, MAURICE E., Ivey Acres, Thiensville
- McKERRROW, GEORGE, 421 N. West Ave., Waukesha
- MEYER, EUGENE C., Assoc. Editor, Hoard's Dairymen, Ft. Atkinson
- MILLER, PAUL G., Carnation Co., Research Lab., Oconomowoc
- NIEDERMEIER, R. P., Dairy Husbandry Dept., Univ. of Wis., Madison
- NUSBAUM, DAVE, c/o L. D. Schreiber Co., 1805 Main St., Green Bay
- OBERG, E. B., 2344 N. Oakland Ave., Milwaukee
- O'DONNELL, E. J., Klenszade Product, Inc., P. O. Box 70, Beloit
- OLSEN, L. S., Olsen Publishing Co., 1445 N. 5th at W. Cherry, Milwaukee 12
- OTTERSON, DONOVAN O., 1620 21st Ave., Monroe
- PECCI, M., Belmonte Cheese Co., 104 S. Main St., Fond Du Lac
- PEPPLER, HENRY J., 2344 N. Oakland, Milwaukee 11
- PHILLIPS, PAUL H., Dept. of Biochemistry, Univ. of Wis., Madison
- POPE, S. M., 1235 Grignon St., Green Bay
- PRATT, SHEERIN E., Westby
- PRENTICE, J. ROCKEFELLER, Lake Geneva
- PRICE, WALTER V., Hiram Smith Hall, Univ. of Wis., Madison
- PROCEP, ARNOLD J., 4129A N. 42nd St., Milwaukee
- PULKRABEK, G. M., 1616 17th St., Monroe
- REEDAL, JOHN, Swiss Tom Farms, Inc., Beloit
- RYDZEWSKI, GEORGE S., 1605 S. 37th St., Milwaukee
- SCHMIT, RAY, 2206 N. 30th St., Milwaukee
- SCOTT, H. T., Walnut St., Box 2059, Madison 5
- SHOGEN, C. B., Klenszade Products, Inc., Beloit
- SLEMMONS, WILBERT S., Carnation Co., Oconomowoc
- SMITH, DONALD I., Milprint, Inc., W. Deperere
- SMITH, VEARL, Dept. of Dairy Husbandry, Univ. of Wis., Madison
- SNELL, CARL H., 903 N. Wilson Ave., Rice Lake
- SNYDER, WALTER E., Route 71, c/o F. C. Middleton, Madison
- SOMMER, H. H., Dept. of Dairy Husbandry, Univ. of Wis., Madison
- SPITZER, ROBERT R., Murphy Products Co., Burlington
- SPRAGUE, GORDON W., 935 E. John St., Appleton
- SPURGEON, KENNETH R., Dept. of Dairy Industry, Univ. of Wis., Madison
- STALLARD, J. E., 207 King Hall, Univ. of Wis., Madison
- STOCKELBERG, FRED, Lode
- STEIN, BRUNO, Shorewood Hills, Madison

STICHA, A. J., 113 W. Lakeside St., Madison 5
 STONE, WARREN K., 2406 Coolidge St., Madison
 SWANSON, ARTHUR M., Dairy Industry Dept., Univ. of Wis., Madison
 TARDOW, LEONARD, 427 W. National Ave., Milwaukee 4
 THEW, HARVEY E., 29 Coyne Court, Madison Milk Prod. Ass'n., Madison
 THOMAS, HUGH, 2525 Major Ave., Madison 4
 THOMPSON, DONALD I., Bloomer
 THOMSEN, L. C., 3414 Viburnum Drive, Shorwood Hills, Madison 5
 TOOTH, GEORGE, Box D, River Falls
 TRISH, KARL A., 7722 6th Ave., Kenosha
 USKELMAN, W. E., 345 Sheboygan St., Fond Du Lac
 VERGERONT, GLEN W., 207 King Hall, College of Agr., Univ. of Wis., Madison
 WAKEMAN, ALDEN N., Lake Mills
 WALLENFELDT, E., Dept. of Dairy Industry, Univ. of Wis., Madison
 WEBER, HIRAM R., 136 5th Ave., Antigo
 WECKEL, KENNETH G., Dept. of Dairy Industry, Univ. of Wis., Madison
 WEIBEL, WILLIAM B., Ellsworth Creamery, Ellsworth
 WEISNER, MILTON, 935 E. John St., Appleton
 WEISSERT, C. D., c/o Schlosser Creamery, Plymouth
 WERNER, GEORGE M., Dairy Husbandry Dept., College of Agr., Madison
 WILKE, THEODORE M., 3167 N. Booth St., Milwaukee 12
 WILLARD, ESTHER M., 330 Goldsmith Bldg., Milwaukee 2
 WILLETT, E. L., American Foundation for the Study of Genetics, R. F. D. 5, Madison
 WOELFFER, E. A., Pabst Farms, Inc., Oconomowoc
 WOODARD, L. O., 1910 Loev St., Appleton
 ZIMMERMAN, PAUL L., c/o Marathon Corp., Research Lab., Menasha

Student Affiliates:

ADAMS, H. P., Dept. of Dairy Husbandry, Univ. of Wis., Madison
 ALBRECHT, T. W., Dept. of Dairy Industry, Univ. of Wis., Madison
 ANDERSON, G. E., 443 Midvale Blvd., Madison 5
 AYTAC, MEHMET, 231 W. Gilman, Madison
 BEEG, LESLIE E., Dept. of Dairy Industry, Univ. of Wis., Madison
 BLACK, WALLACE G., Dept. of Genetics, Univ. of Wis., Madison
 BLOCK, ALTON DALE, Juneau
 BOND, WILLIAM H., Room 103, King Hall, Univ. of Wis., Madison
 BRYE, FREDERICK A., 603 S. Main St., Viroqua
 BUSH, ROBERT G., 2335 Du Charme Lane, Green Bay

CARCEY, JOSEPH W., 305 N. Warren St., Watertown
 CARPENTER, ROMAN A., 2224 Regent St., Madison 5
 CHAN, KIN-MAN, Dept. of Dairy Industry, Univ. of Wis., Madison
 CHESLEY, RICHARD C., 7932 Harwood Ave., Wauwatosa 13
 CORLEY, E. L., 1912 West Lawn Ave., Madison 5
 CROWLEY, JAMES W., Dept. of Dairy Husbandry, Univ. of Wis., Madison
 DAINES, TOM, 115 W. Wilson St., Madison
 HANSEN, LEO J., Route 3, Box 3, White-water
 HELDEKE, B. E., 34 Highland St., Rice Lake
 HUSTON, KEITH, Dept. of Dairy Husbandry, Univ. of Wis., Madison
 JAFAR, SYED M., Dept. of Genetics, Univ. of Wis., Madison
 KARAM, HASSAN A., Dept. of Genetics, Univ. of Wis., Madison
 KAUTZ, HERMAN G., Dept. of Dairy Industry, Hiram Smith Hall, Univ. of Wis., Madison
 KOPENHAVER, JOSEPH A., 403 Academy St., Elroy
 LINDEN, HAROLD K., 558, Badger
 LISS, RALPH M., R. E. 1, Hartland
 MARION, GERMAIN B., Dept. of Dairy Husbandry, Univ. of Wis., Madison
 MAXCY, R. B., Dept. of Dairy Industry, Univ. of Wis., Madison
 MEHRING, EUGENE F., 115 W. Wilson St., Madison
 MILLER, J. T., Dept. of Dairy Husbandry, Univ. of Wis., Madison
 MITCHELL, JOHN T., County Dairy Agent, Shawano
 MOSTAFA, AHMAD M., Dept. of Dairy Industry, Univ. of Wis., Madison
 O'NEILL, NESTOR COLON, Dept. of Dairy Husbandry, Univ. of Wis., Madison
 REED, BLAKE L., Dept. of Dairy Husbandry, Univ. of Wis., Madison
 SCHMIDT, ROBERT S., R. F. D. 1, Burnett
 SCHULTZ, LORIS H., Dept. of Dairy Industry, Univ. of Wis., Madison
 SHARRATT, ROBERT H., T Carnation Company, Jefferson
 SINGH, CHANDRA BHAN, Dairy Industry Dept., Univ. of Wis., Madison
 STEINER, BURTON, 1017 17th St., Monroe
 STOLL, ROBERT L., Route 1, Osseo
 TORO, EMILIO DEL, Dept. of Dairy Husbandry, Univ. of Wis., Madison
 TRIVEDY, JAI NABAIN, Dairy Husbandry Dept., Univ. of Wis., Madison
 TYZNIK, WILLIAM, Dept. of Dairy Husbandry, Univ. of Wis., Madison
 VAN VOORHEES, DAVID J., Redgranite
 WILEY, THOMAS, 3702 Hillcrest Drive, Madison
 WINDER, WILLIAM C., Dept. of Dairy Industry, Univ. of Wis., Madison
 WREND, G., Middleton

WYOMING

Members:

BROWN, DONALD C., Animal Prod. Dept.,
Univ. of Wyo., Laramie
CARTER, HUGH C., Laramie Valley Cream-
ery, 166 N. 3rd St., Laramie

ENGENDORFF, O. H., State Dept. of Agr.,
Box 17, Worland
HAMS, RAY E., 51 Bellvue Ave., Sheridan
LOUGHARY, IVAN H., Extension Dairyman,
Univ. of Wyo., Laramie
WILLARD, H. S., Box 791, Laramie

AUTHOR INDEX OF ORIGINAL ARTICLES

- ACKERMAN, R. A.**, prepartum production and composition of colostrum, 559; prepartum milking and performance, 719*; prepartum milking and colostrum composition, 720*
- AGEE, C. B.**, cultured ice cream, 697*
- ALBRECHTSEN, R.**, I.B.M. equipment for processing BDI 718 reports, 728*; production record analysis for daughters of sires in artificial insemination program, 730*
- ALEXANDER, M. H.**, methods for estimating production, 621
- ALLEN, N. N.**, clipping for louse control, 724*
- ALLEN, R. S.**, soybeans and vitamin A and carotene in plasma and milk, 565; soybean oils vs. butter oil for calves, 609; interference with activated glycerol dichlorohydrin test, 688; effect of carotenoid dispersion on absorption, 711*
- ALMQUIST, J. O.**, penicillin and streptomycin in semen, 183; bacteria and semen fertility, 543; antibiotics and semen fertility, 722*; eosin-aniline blue stain for semen, 723*; bacteria and semen fertility, 849; penicillin and semen fertility, 950
- AMERICAN DAIRY SCIENCE ASSOCIATION**, call for papers for annual meeting, 96; officers and committees, 96; registration and housing for 44th annual meeting, 381; program for 44th annual meeting, 477; proceedings of 44th annual meeting, 731
- ANDERSON, E. O.**, leucocyte count of milk, 649; leucocyte count of postpartum samples, 804
- ARBUCKLE, W. S.**, measuring ice cream structure, 696*
- ARCHIBALD, J. G.**, nickel in cows' milk, 877; nutrient composition of banana skins, 969
- ARRINGTON, L. R.**, freezing point of milk, 699*
- ASDELL, S. A.**, review on nutrition and treatment of sterility, 60; review on hormones for treatment of sterility, 45
- ASHWORTH, U. S.**, turbidity and homogenization, 698*
- ATKINSON, F. W.**, vitamin A and carotenoids of colostrum, 209; milking procedures and mammary secretion composition, 719*; mammary edema and roughage feeding, 721*
- BABCOCK, O. J.**, stabilizers in frozen homogenized milk, 175; milk standards, 703*; agitation while freezing homogenized milk, 812
- BABEL, F. J.**, bacteriophage in lactic cultures, 702*; dye reduction tests, 702*
- BAKER, F. N.**, testosterone and oxygen consumption of spermatozoa, 726*
- BAKER, R. J.**, amino acid addition to Cheddar cheese, 769
- BALTZER, BETTY**, *Brucella abortus* destruction in home pasteurizers, 29
- BAKER, H. B.**, fat concentrations in blood plasma, 709*
- BARTLETT, DAVID E.**, review on infertility caused by infectious disease, 36
- BARTLEY, E. E.**, unsaturation of milk fat, 700*
- BASNETT, R. J.**, whey solids in ice cream, 697*
- BECKER, R. R.**, protozoa in bovine stomach, 806
- BELL, R. W.**, oxidized flavor in frozen milk, 699*; stability of ascorbic acid, 699*; ascorbic acid stability in water, 827; ascorbic acid and oxidized flavor, 833
- BENDER, C. B.**, energy sources for silage organisms, 901
- BIRD, E. W.**, unsaturation of milk fat, 700*
- BLACK, A. L.**, distribution of injected P³², 717*
- BLANTON, L. F.**, measuring ice cream structure, 696*
- BLOSSER, T. H.**, wood molasses as feed, 719*
- BOESTEDT, G.**, fat and vitamin A in sow's milk, 22
- BOWLAND, J. P.**, fat and vitamin A in sow's milk, 22
- BOYD, W. L.**, vitamin E for cattle, 495
- BOYER, P. D.**, vitamin E for cattle, 495
- BRANTON, CECIL**, plant and animal proteins for bulls, 292; semen dilution, 721*; frequency of semen collection, 723*
- BRATTON, R. W.**, plant and animal proteins for bulls, 292; oxygen damage to spermatozoa, 853; diluents for semen, 604; buffered egg as semen extender, 722*; cooling semen with and without extender, 723*, 856
- BRODY, S.**, cow reaction to high temperatures, 718*

* Abstract of paper presented at annual meeting.

- BROWN, K., vitamin A in dried milk, 695*
- BROWN, R. W., Canadian Cheddar cheese from pasteurized milk, 1
- BRUNNER, J. R., solidification of fat in cream, 695*
- BUCKNER, CECILIA R., mastitis organisms, 720*; penicillin for mastitis, 720*
- BURCH, B. J., JR., sweet potatoes as feed, 725*
- BURKEY, L. A., mastitis organisms, 720*; penicillin for mastitis, 720*
- BYERS, J. H., feeding high and low fat concentrates, 596
- CALBERT, HAROLD E., diacetyl and flavor of Cheddar cheese, 515; changes in diacetyl content of cheese, 521
- CALVERLY, C. E., vitamin E for cattle, 495
- CANNON, C. Y., soybean oil vs. milk fat for calves, 429; soybeans and vitamin A and carotene in plasma and milk, 565; unsaturation of milk fat, 700*
- CARTER, R. H., feed and DDT in cow's milk, 549
- CARY, C. A., role and sources of B₁₂, 715*
- CASADY, R. B., excretion of fecal androgens by the cow, 265
- CASIDA, L. E., reproductive failures in cows, 237
- CHAPMAN, A. B., inbred and outbred Holsteins, 247
- CHILSON, W. H., ascorbic acid and oxidized flavor in milk, 306; surface tension of milk, 698*
- CHITWOOD, T., riboflavin in chocolate milk, 823
- CHOI, R. P., solubility method for alpha and beta lactose, 391; determination of color of dry milk, 580
- CLAYDON, T. J., segregation vs. mixing of cream, 534; cream in buying stations, 935
- CLONINGER, W. H., 4-H dairy project leader training, 729*
- COBBLE, J. W., temperature and milk composition, 713*
- COLL, H. H., excretion of fecal androgens by the cow, 265
- COLLINS, E. B., bacteria and soluble nitrogen of milk, 652; bacteriophage multiplication, 701*
- COLOVOS, N. F., nutritive value of timothy hay, 659; nutritive value of wood molasses, 907
- COMBS, W. B., dry buttermilk in bread, 703*; surface growth on blue cheese, 704*
- CONKLIN, C. T., age and type ratings of Ayrshire females, 375
- CONRAD, RALPH M., surface tension of fresh surfaces of milk, 406
- CONVERSE, H. T., vitamin A and carotene intake and depletion of calves, 418
- COOK, GLEN L., surface tension of fresh surfaces of milk, 406
- COULTER, S. T., reducing systems in dry whole milk, 334; dry buttermilk in bread, 703*; surface growth on blue cheese, 704*
- CRISLER, O. S., mastitis diagnosis and therapy, 721*
- DAHLBERG, A. C., ripening temperature and tyramine content of cheese, 316; cheese made with *S. faecalis*, 630; phosphatase test for cheese, 751; buffer for cheese phosphatase test, 760; radio-frequency pasteurization of cheese, 790
- DAHLE, C. D., fatty fractions of butter oil, 700*
- DAVIS, H. P., thyroxine and semen fertility, 322; testosterone and oxygen consumption of spermatozoa, 726*
- DAWDY, M. L., prepartum milking and milk production, 725*
- DEXTER, S. T., influence of soil fertility, 716*
- DEYSHER, E. F., measurement of viscosity of dairy products, 452
- DICKE, R. J., clipping for louse control, 724*
- DICKERSON, G. E., inbred and outbred Holsteins, 247
- DRACY, A. E., recovery of fertilized bovine ovum, 726*
- DRYDEN, L. P., role and sources of B₁₂, 715*
- DUNCAN, C. W., corn supplement for all-falfa ration, 465; amino acid content of colostrum and milk, 671; influence of soil fertility, 716*
- DUNN, H. O., buffered egg as semen extender, 722*
- DUNN, K. M., influence of soil fertility, 716*
- EATON, H. D., prepartum vitamin A supplementation and calf health, 367; composition of first colostrum, 587; pH of calf feces, 709*; prepartum milking and calf plasma carotenoids, 711*; fetal stores of vitamin A, 764; prepartum milking and

- blood constituents, 870; effect of milking interruption, 977; prepartum milking and calf plasma levels of carotenoids, 1032; prepartum milking and colostrum composition, 919
- EDMONDSON, J. H., interval between parturition and estrus, 726*
- EDMONSON, J. E., mastitis diagnosis and therapy, 721*
- ELDERIDGE, FRANKLIN E., pedigree promise and proved performance of bulls, 841
- ELLIOTT, R. F., carotene requirements of calves, 710*; carotene absorption and conversion, 711*
- ELMS, G. H., cobalt tolerance in cattle, 527
- ELLMORE, M. F., soybeans and vitamin A requirements, 714*
- ELY, F., interrelationship of age and season on bull fertility, 727*
- ELY, R. E., amino acid content of colostrum and milk, 671; forage preservation procedures, 716*; influence of soil fertility, 716*
- EMERY, H. J., milk standards, 703*
- EEB, R. E., wood molasses as feed, 719*; centering date vs. calendar month for records, 728*
- EERIKSON, A. B., free tryptophane in cheese, 704*
- F** AIRBANKS, B. W., solubility method for alpha and beta lactose, 391; determination of color of dry milk, 580
- FISCHER, JESSIE E., review on lactose and gastro-intestinal motility, 139; lactose feeding and lactase production, 717
- FISHMAN, R. H., vitamin D in roughages, 715*
- FLANDERS, C. A., prepartum milking and colostrum composition, 720*
- FLORA, C. C., milk handling and bacterial content, 724*
- FOHRMAN, M. H., Jersey growth standards, 718*
- FOOTE, R. H., diluents for semen, 604; cooling semen with and without extender, 723*; semen cooling with and without extender, 856
- FORD, H. F., bacteriophage in lactic cultures, 702*
- FORD, T. F., colloidal protein of skim milk, 697*
- FOSTER, H. G., milk phosphatase inactivation rate, 509
- FOUNTAIN, F. C., milking procedures and mammary secretion composition, 719*; mammary edema and roughage feeding, 721*
- FRANCIS, J., oxygen content of milk, 202
- FRAZER, W. C., yeast on brick cheese, 475
- FRYE, J. B., JR., sweet potatoes as feed, 725*
- FYOCK, W. D., dehydrated banana meal in calf rations, 361
- G** ALLUP, W. D., carotene and reproduction, 714*
- GEARHART, C. R., D.H.I.A. computing tables, 727*
- GEHRKE, C. W., riboflavin in chocolate milk, 823
- GOLSON, J. H., cultured ice cream, 697*; whey solids in ice cream, 697*
- GILMORE, H. C., I.B.M. machines for processing D.H.I.A. records, 728*
- GILMORE, LESTER O., review on inheritance of reproductive inefficiency, 71; calf mortality, sex ratio and twinning, 706*
- GLANTZ, P. J., penicillin and streptomycin in semen, 183
- GOLDING, N. S., oxygen requirements for molds, 101
- GOULD, I. A., solvent extraction vs. churning for lipase activity measurement, 435; solvent extraction procedure for fat for titration, 447
- GREENBANK, G. R., ascorbic acid in dried milk, 644; keeping quality of dried milk, 696*
- GREGORY, P. W., prolonged gestation in cattle, 705*
- GRUBER, G. W., vitamin A in dried milk, 695*
- GULLICKSON, T. W., vitamin E for cattle, 495
- GUMMER, R. H., fat and vitamin A in sow's milk, 22
- GUTHRIE, E. S., stability of fat and fat-soluble vitamins, 679; body of cultured cream, 695*; vitamin C elimination from milk, 786
- H** AAG, J. R., feeding high and low fat concentrates, 596
- HAGDAHL, L., chromatographic separation of fatty acids, 700*
- HALVORSEN, H. O., coliform thermal death times, 702*
- HADJIOLOS, B. C., ketosis studies, 718*
- HANCKEL, R. M., cultured ice cream, 697*; whey solids in ice cream, 697*

- HARLAND, H. A., reducing systems in dry whole milk, 334
- HARSHBARGER, K. E., roughages and growth rates, 715*; time required for feed consumption, 716*
- HART, G. H., excretion of fecal androgens by the cow, 265
- HARTMAN, A. M., role and source of B₁₂, 715*
- HAYNES, (MRS.) V. M., dry milk in bread, 703*
- HELMBOLDT, C. F., parturition milking and blood constituents, 870
- HENDERSON, C. B., herd environment changes, 706*
- HENDERSON, H. O., pasteurized milk in home refrigerators, 945
- HENKE, L. A., excess concentrate feeding in Hawaii, 725*
- HERMAN, H. A., mastitis diagnosis and therapy, 721*; pH of bovine vaginal-cervical mucus, 727*
- HERREID, E. O., oxygen content of milk, 202; nordihydroguaiaretic acid in sweetened frozen cream, 301; lyophilizing apparatus, 973
- HERRINGTON, B. L., radio-frequency pasteurization of cheese, 790
- HETRICK, J. H., nitrogen in milk by nesslerization, 111
- HIBBS, J. W., influence of early rumen development on blood constituents, 710*; rumen microflora, 710*; ration and blood composition, 1016; establishment of rumen microorganisms, 1025; pasture and carotenoid content of milk, 955
- HIGGINS, L. A., Mississippi 4-H show program, 729*
- HILDER, R. A., heat tolerance of dairy cattle, 712*; heat tolerance of calves, 712*
- HILKER, L. D., corn sweeteners in ice cream, 697*
- HOLDWAY, C. W., fused tricalcium phosphate for dairy cattle, 228; milk handling and bacterial content, 724*
- HOLMAN, R. T., chromatographic separation of fatty acids, 700*
- HOLMES, ARTHUR D., ascorbic acid loss from lactose-enriched milk, 556
- HOOD, E. G., antibiotic action on lactic streptococci, 961
- HORWOOD, R. E., Michigan brucellosis control, 729*
- HOSTERMAN, W. H., forage preservation procedures, 716*
- HUDDLESON, I. FOREST, *Brucella abortus* destruction in home pasteurizers, 29
- HUFFMAN, C. F., properties of bovine saliva, 123; corn supplement for all-alfalfa ration, 465
- HUGHES, J. S., vitamin A and carotenoids of colostrum, 209; colostrum vitamin A and carotenoids, 458; milking procedures and mammary secretion composition, 719*
- HYATT, GEORGE, JR., age and type ratings of Ayreshire females, 375; parturition production and composition of colostrum, 559; differences in cow families, 705*; parturition milking and performance, 719*
- ITSCHNER, E. T., 4-H dairy project leader training, 729*
- IYA, K. K., yeast on brick cheese, 475
- JACK, E. L., dry milk in bread, 703*; solidification of fat in cream, 695*
- JACOBSON, N. L., soybean oil vs. milk fat for calves, 429; soybean oils vs. butter oil for calves, 609; interference with activated glycerol dichlorohydrin test, 688; fat concentrations in blood plasma, 709*; effect of carotenoid dispersion on absorption, 711*
- JACOBSON, W. C., vitamin A and carotene intake and depletion of calves, 418
- JANZEN, J. J., Canadian Cheddar cheese from pasteurized milk, 1
- JARVIS, R. N., cottonseed oils for calf feeding, 665
- JENNESS, ROBERT, reducing systems in dry whole milk, 334; reducing groups in milk, 698*
- JOHNSON, B. C., solvent extraction vs. churning for lipase activity measurement, 435; solvent extraction procedure for fat for titration, 447
- JOHNSON, MARVIN J., fatty acid liberation in Cheddar cheese, 862
- JOHNSON, R. E., composition of first colostrum, 587; pH of calf feces, 709*; parturition milking and calf plasma carotenoids, 711*; fetal stores of vitamin A, 764; parturition milking and blood constituents, 870; effect of milking interruption, 977; parturition milking and calf plasma levels of carotenoids, 1032; parturition milking and colostrum composition, 919
- JOHNSTON, JAMES E., hyaluronidase assay of

- bull semen, 570; hyaluronidase in bull semen, 574
- JONES, E. R., JR., protozoa in bovine stomach, 806
- JONES, I. R., feeding high and low fat concentrates, 596
- JOSEPHSON, DONALD V., furfuryl alcohol from heated milk, 222; nitroprusside test on heated milk, 398
- JUNGHEER, E. L., pH of calf feces, 709*; prepartum milking and blood constituents, 870
- K**KATZNELSON, H., antibiotic action on lactic streptococci, 961
- KEENER, H. A., nutritive value of timothy hay, 659; cobalt tolerance in young cattle, 527; nutritive value of wood molasses, 907
- KEENEY, D. G., organic compounds from heated milk, 698*
- KENDRICK, J. F., D.H.I.A. herdbook revision suggestions, 727*
- KESLER, E. M., interval between washing udder and milking, 637
- KLEIBER, MAX, distribution of injected P³², 717*; venous catheterization, 889
- KNOBT, C. B., extended thyroprotein feeding, 257; dehydrated banana meal in calf rations, 361; interval between washing udder and milking, 637; milk replacements in calf rations, 709*, 986; prepartum milking and milk production, 725*
- KNOOP, C. E., let-down of milk, 725*
- KOCH, R. B., dry buttermilk in bread, 703*
- KONCUS, A. F., determination of color of dry milk, 580
- KOSIKOWSKY, F. V., ripening temperature and tyramine content of cheese, 316; cheese made with *S. faecalis*, 630; amino acids and amines in cheese, 705*; cheese phosphatase test, 751; buffer for cheese phosphatase test, 760; radio-frequency pasteurization of cheese, 790
- KOZLIK, R., vitamin A in dried milk, 695*
- KRAMER, J. H., pH of calf feces, 709*; prepartum milking and blood constituents, 870
- KRAUSS, W. E., pasture and carotenoid content of milk, 955
- KRIENKE, W. A., electrometric titration of milk, 699*; freezing point of milk, 699*
- KRUKOVSKY, VLADIMIR, N., influence of tocopherols and cod-liver oil on production, 133; ascorbic acid oxidation in milk, 163; influence of tocopherols and cod-liver oil on milk stability, 196; stability of fat and fat-soluble vitamins, 679; nordihydroguaiaretic acid in cream, 695*; tocopherol in milk fat, 700*; vitamin C elimination from milk, 786
- KUHLMAN, A. H., carotene and reproduction, 714*
- L**LARDY, H. A., blood sugar and ketosis, 718*; blood ketone levels of ruminants, 817
- LARSON, B., reducing groups in milk, 698*
- LAWRENCE, J. L., lactose feeding and lactase production, 717*
- LEAR, S. A., milk phosphatase inactivation rate, 509
- LEFFEL, E. C., ketosis studies, 718*
- LEWTON, R. K., dye reduction tests, 702*
- LIU, T. Y., metabolism of lactogenic hormone, 881
- LOOSLI, J. K., influence of tocopherols and cod-liver oil on production, 133; influence of tocopherols and cod-liver oil on milk stability, 196; prepartum vitamin A supplementation and calf health, 367; tocopherol in milk fat, 700*; milk substitutes for calves, 709*
- LUDWICK, T. M., interrelationship of age and season on bull fertility, 727*
- LUECKE, R. W., amino acid content of colostrum and milk, 671
- LUNDQUIST, NORMAN S., vaccination with *Brucella abortus* (strain 19), 914
- M**MACLEOD, PATRICIA, leucocyte count of milk, 649; leucocyte count of postpartum samples, 804
- MACY, H., coliform thermal death times, 702*
- MAQSOOD, M., temperature and thyroid status, 712*
- MARKLAND, D. M., dye reduction tests, 702*
- MARTIN, W. H., ascorbic acid and oxidized flavor in milk, 306; segregation vs. mixing of cream, 534; cream in buying stations, 935
- MATHEE, R. E., fused tricalcium phosphate for dairy cattle, 228; energy sources for silage organisms, 901
- MATTHESON, L. D., composition of first colostrum, 587; prepartum milking and calf plasma carotenoids, 711*; fetal stores of vitamin A, 764; prepartum milking and blood constituents, 870; effect of milking

- interruption, 977; prepartum milking and calf plasma levels of carotenoids, 1032; prepartum milking and colostrum composition, 919
- MATTHEWS, C. A., thyroid size, 708*; Jersey growth standards, 718*
- MCDOWELL, R. E., heat tolerance of cattle, 712*; heat tolerance of calves, 712*
- MEAD, S. W., prolonged gestation in cattle, 705*
- MELIN, C. G., forage preservation procedures, 716*
- MERCER, D. N., effect of milking interruption, 977
- MERILAN, C. P., mastitis diagnosis and therapy, 721*
- MILLAR, C. E., influence of soil fertility, 716*
- MILLER, D. D., oxygen requirements for molds, 101
- MILLER, G. D., sweet potatoes as feed, 725*
- MILLER, K., calf mortality, sex ratio and twinning, 706*
- MITCHELL, H. L., carotene determination in silage, 33
- MIXNER, JOHN P., hyaluronidase assay of bull semen, 570; hyaluronidase in bull semen, 574; penicillin and sulfanilamide in semen dilutors, 722*; triphenyltetrazolium chloride for semen studies, 1013
- MONROE, C. F., let-down of milk, 725*; pasture and carotenoid content of milk, 955
- MOORE, L. A., thyroprotein feeding during first lactation, 278; testis biopsy of bulls, 327; vitamin A and carotene intake and depletion calves, 418; feed on DDT in cow's milk, 549
- MORRIS, H. A., surface growth on blue cheese, 704*
- MORRISON, R., centering date vs. calendar month for records, 728*
- MORROW, K. S., cobalt tolerance in young cattle, 527
- MUCHA, T. J., oxidized flavor in frozen milk, 699*; stability of ascorbic acid, 699*; ascorbic acid stability in water, 827; ascorbic acid and oxidized flavor, 833
- MUELLER, W. S., quaternary germicides, 703*
- MURLEY, W. R., soybean oils vs. butter oil for calves, 609
- MUSGRAVE, S. D., diluents for semen, 604
- MUSSETT, A. T., fat fractions of butter oil, 700*
- NELSON, F. E., bacteria and soluble nitrogen of milk, 652; bacteriophage multiplication, 701*; pasteurized milk for blue cheese, 704*; amino acid addition to Cheddar cheese, 769; flavor of blue cheese, 1007; *Candida lipolytica* in blue cheese, 993; mold-enzyme preparation for blue cheese, 1001
- NELSON, J. W., vitamin E for cattle, 495
- NELSON V., physical properties of evaporated milk, 775
- NEVENS, W. B., feed-replacement value of pasture, 717*; feed-replacement value of pasture, 894
- NEWSOM, M. H., semen dilution by numbers, 721*; frequency of semen collection, 723*
- NEZVESKY, L., pH of calf feces, 709*; effect of milking interruption, 977; prepartum milking and calf plasma levels of carotenoids, 1032; prepartum milking and colostrum composition, 919
- NIEDERMEIER, R. P., vitamin A absorption, 714*; blood constituents of mastectomized cows, 927
- OLSON, F. C., vitamin E for cattle, 495; vitamin A in dried milk, 695*
- OLSON, J. C., JR., coliform thermal death times, 702*; coliform bacteria from mastitis, 721*
- O'MALLEY, C. M., solubility method for alpha and beta lactose, 391; determination of color of dry milk, 580
- ORMISTON, E. E., calf losses, 712*
- FALMER, L. S., vitamin E for cattle, 495
- PARFITT, E. H., sanitary standardization of dairy equipment, 701*
- PARKIN, I. E., milking machine operation, 728*
- PARMELEE, C. E., bacteriophage multiplication, 701*; dry milk in cottage cheese, 705*; *Candida lipolytica* in blue cheese, 993; flavor of blue cheese, 1007; mold-enzyme preparation for blue cheese, 1001
- PARRISH, D. B., carotene determination in silage, 33; vitamin A and carotenoids of colostrum, 209; ascorbic acid and oxidized flavor in milk, 306; colostrum vitamin A and carotenoids, 458; milking procedures and mammary secretion composition, 719*; mammary edema and roughage feeding, 721*

- PATTON, STUART, furfuryl alcohol from heated milk, 222; nitroprusside test on heated milk, 398; organic compounds from heated milk, 698*; fatty fractions of butter oil, 700*
- PATRICK, T. E., semen dilution by numbers, 721*; frequency of semen collection, 723*
- PAULSON, KATHERINE, stale-flavor of butter, 701*
- PERCIVAL, G. P., cobalt tolerance in young cattle, 527
- PETERS, I. I., pasteurized milk for blue cheese, 704*
- PETERSEN, W. E., recovery of fertilized bovine ovum, 726*
- PETERSON, MERLIN H., fatty acid liberation in Cheddar cheese, 862
- PHILLIPS, P. H., fat and vitamin A in sow's milk, 22; vaccination with *Brucella abortus* (strain 19), 914
- PLASTRIDGE, W. N., pH of calf feces, 709*; effect of milking interruption, 977
- POOS, F. W., feed and DDT in cow's milk, 549
- POTTER, F. E., measurement of viscosity of dairy products, 452; whey in sherbets, 696*
- POUNDEN, W. D., influence of early rumen development on blood constituents, 710*; rumen microflora, 710*; establishment of rumen microorganisms, 1025; ration and blood composition, 1016
- PRATT, A. D., fused tricalcium phosphate for dairy cattle, 228
- PRESCOTT, J. A., JR., feed-replacement value of pasture, 717*; feed-replacement value of pasture, 894
- PRESCOTT, J. H., nutritive value of timothy hay, 659
- PRESCOTT, J. R., nutritive value of wood molasses, 907
- PRICE, R. B., JR., clipping for louse control, 724*
- PRICE, WALTER V., diacetyl flavor of Cheddar cheese, 515; changes in diacetyl content of cheese, 521; fatty acid liberation in Cheddar cheese, 862
- PRIGGE, W. C., clipping for louse control, 724*
- PRINCE, P. W., bacteria and semen fertility, 543; bacteria and semen fertility, 849
- PYENSON, H., emulsifying agents in ice cream, 696*
- RAGSDALE, A. C., temperature and carotenoids of milk fat, 713*; temperature and milk composition, 713*
- RALSTON, N. P., distribution of injected P³², 717*; venous catheterization, 889
- RAMSEDELL, G. A., colloidal protein of skim milk, 697*
- RANDALL, RAYMOND, stabilizers in frozen homogenized milk, 175; agitation while freezing homogenized milk, 812
- REAVES, P. M., milk handling and bacterial content, 724*
- REGAN, M. J., 4-H dairy project leader training, 729*
- REGAN, W. M., prolonged gestation in cattle, 705*
- REGER, J. V., dry buttermilk in bread, 703*
- REID, J. J., bacteria and semen fertility, 543; interval between washing udder and milking, 637; bacteria and semen fertility, 849
- REID, J. T., properties of bovine saliva, 123
- REID, W. H. E., cultured ice cream, 697*; whey solids in ice cream, 697*
- REINEKE, E. P., temperature and thyroid status, 712*
- RIDDELL, W. H., European dairy production, 724*
- ROARK, D. B., pH of bovine vaginal-cervical mucus, 727*
- ROSENBERGER, J., interrelationship of age and season on bull fertility, 727*
- ROSENBERGER, W. S., dry milk in cottage cheese, 705*
- RUDRAIAH, D. S., interrelationship of age and season on bull fertility, 727*
- RUSOFF, L. L., sweet potatoes as feed, 725*
- SAARINEN, P. V., ketosis studies, 718*
- SAGER, OSCAR S., sample preservation for phosphatase test, 166
- SALISBURY, G. W., plant and animal proteins for bulls, 292; oxygen damage to spermatozoa, 353; roughages and growth rates, 715*; pedigree promise and proved performance of bulls, 841
- SALSBUURY, R. L., energy sources for silage organisms, 901
- SANDERS, GEORGE P., sample preservation for phosphatase test, 166
- SARKAR, B. C. RAY., amino acid content of colostrum and milk, 671
- SCHEIDENHELM, E. C., integrated dairy program, 729*
- SCHIPPER, I. A., coliform bacteria from mastitis, 721*

- SCHMITZ, M. E., coliform bacteria from mastitis, 721*
- SCHOENLEBER, L. G., forage preservation procedures, 716*
- SCHULTZ, L. H., vitamin A absorption, 714*; blood sugar and ketosis, 718*; blood ketone levels of ruminants, 817
- SCHULTZE, A. B., thyroxine and semen fertility, 322; acetylcholine+thyroxine and O₂ consumption of semen, 726*; testosterone and oxygen consumption of spermatozoa, 726*
- SCOTT, G. W., wood molasses as feed, 719*
- SEATH, D. M., hay for cows on pasture, 717*
- SEELEY, D. R., quaternary germicides, 703*
- SHAFFER, H. E., penicillin and streptomycin effect in semen, 183; eosin-aniline blue stain for semen, 723*
- SHAW, A. O., wood molasses as feed, 719*
- SHAW, J. C., soybeans and vitamin A requirements, 714*; ketosis studies, 718*
- SHEPHERD, J. B., feed and DDT in cow's milk, 549; carotene in feed and vitamin A in butter, 714*; forage preservation procedures, 716*
- SHETLAR, M. R., riboflavin in chocolate milk, 823
- SHEURING, J. J., emulsifying agents in ice cream, 696*; ice cream shrinkage, 697*
- SLATE, R. J., prepartum milking and blood constituents, 870
- SMITH, A. H., distribution of injected P³², 717*; venous catheterization, 889
- SMITH, C. A., vitamin D in roughages, 715*
- SMITH, YEAL R., time of milking after conditioned stimulus, 17; vitamin A absorption, 714*; blood sugar and ketosis, 718*; blood ketone levels of ruminants, 817; blood constituents of mastectomized cows, 927
- SPIELMAN, A. A., prepartum vitamin A supplementation and calf health, 367; composition of first colostrum, 587; prepartum milking and calf plasma carotenoids, 711*; fetal stores of vitamin A, 764; prepartum milking and blood constituents, 870; effect of milking interruption, 977; prepartum milking and calf plasma levels of carotenoids, 1032; prepartum milking and colostrum composition, 919
- SQUIBB, R. L., soybeans and vitamin A and carotene in plasma and milk, 565
- STABILE, JOSEPH N., stabilizers in frozen homogenized milk, 175
- STAHLY, G. L., lactose feeding and lactase production, 717*
- STALLCUP, O. T., temperature and carotenoids of milk fat, 713*
- STULL, J. W., nordihydroguaiaretic acid in sweetened frozen cream, 301; lyophilizing apparatus, 973
- SUTTON, T. S., review on lactose and gastrointestinal motility, 139; lactose feeding and lactase production, 717*
- SWANSON, ERIC W., bull semen diluters, 345; thyroprotein feeding, 708*
- SWANSON, R. G., extended thyroprotein feeding, 257
- SWEETMAN, W. J., testis biopsy of bulls, 327
- SWETT, W. W., thyroid size, 708*; penicillin for mastitis, 720*
- SYKES, J. F., approaching problem of infertility, 92; thyroprotein feeding during first lactation, 278; testis biopsy of bulls, 327; hormone-induced mammary development, 707*
- T**ABLER, K. A., differences in cow families, 705*
- TALLMAN, K. L., mastitis diagnosis and therapy, 721*
- TANABE, T. Y., reproductive failures in cows, 237
- TATTER, C. W., solubility method for alpha and beta lactose, 391
- TEERI, A. E., nutritive value of timothy hay, 659; nutritive value of wood molasses, 907
- THEOKAS, D. A., stability of fat and fat-soluble vitamins, 679; nordihydroguaiaretic acid in cream, 695*; tocopherol in milk fat, 700*
- THOMAS, R. H., soybean oil vs. milk fat for calves, 429
- THOMAS, J. W., thyroprotein feeding during first lactation, 278; heart rates of cows, 708*
- THOMAS, L. C., riboflavin in chocolate milk, 823
- TOUCHBERRY, R. W., feed-replacement value of pasture, 717*, 894
- TRACY, P. H., ash alkalinity of dry butter-milk, 191; nordihydroguaiaretic acid in sweetened frozen cream, 301; stale flavor distribution in dried whole milk, 383; emulsifying agents in ice cream, 696*; extraction of stale butteroil, 701*; stale-flavor of butter oil, 701*

- TAOUT, G. M., *Brucella abortus* destruction in home pasteurizers, 29
- TURK, K. L., influence of tocopherols and cod-liver oil on production, 133; milk substitutes for calves, 709*; prepartum vitamin A supplementation and calf health, 367
- TURNER, C. W., hyperthyroidism and milk production, 707*; male hormone in cow manure, 707*; activation of male hormone in cow manure, 796; metabolism of lactogenic hormone, 881
- TYLER, W. J., inbred and outbred Holsteins, 247; age and type ratings of Ayrshire females, 375; differences in cow families, 705*; transmitting ability of a sire, 706*
- U**NDERWOOD, P. C., testis biopsy of bulls, 327; hormone-induced mammary development, 707*; penicillin for mastitis, 720*
- UZZELL, E. M., protozoa in bovine stomach, 806
- V**ANDEMARK, N. L., oxygen damage to spermatozoa, 353; diluents for semen, 604
- VAN LANDINGHAM, A. H., prepartum production and composition of colostrum, 559; prepartum milking and performance, 719*; prepartum milking and colostrum composition, 720*
- W**AGNER, R. E., forage preservation procedures, 716*
- WALLACE, H. D., milk substitutes for calves, 709*
- WALLIS, G. C., vitamin D in roughages, 715*
- WARD, GERALD M., time of milking after conditioned stimulus, 17
- WASHBON, W. E., sire selection, 730*; transmitting ability of a sire, 706*
- WAUGH, R. K., cottonseed oil for calf feeding, 665
- WEAKLEY, C. E., JR., prepartum production and composition of colostrum, 559
- WEBB, B. H., measurement of viscosity of dairy products, 452
- WEESE, S. J., pasteurized milk in home refrigerators, 945
- WEISER, H. H., lactose feeding and lactase production, 717*
- WHITEHAIR, C. K., blood constituents of mastectomized cows, 927
- WHITING, FRANK, influence of tocopherols and cod-liver oil on production, 133; influence of tocopherols and cod-liver oil on milk stability, 196; stability of fat and fat-soluble vitamins, 679; nordihydroguaiaretic acid in cream, 695*
- WHITMAN, D. W., ash alkalinity of dry buttermilk, 191
- WHITNAH, CARRELL H., surface tension of fresh surfaces of milk, 406; surface tension of milk, 698*
- WHITNEY, R. M., nitrogen in milk by nesslerization, 111
- WHITNEY, R. McL., stale flavor distribution in dried whole milk, 383; extraction of stale butteroil, 701*; stale-flavor of butter oil, 701*
- WILLARD, H. S., standardization of hay palatability trials, 10
- WILLETT, E. L., high dilutions of semen, 722*
- WILLIAMS, D. H., whey in sherbets, 696*
- WILLIAMS, J. B., milk replacements in calf rations, 709*; milk replacements for calves, 986
- WILLIAMS, P. S., interval between washing udder and milking, 637
- WINDHAM, ERNEST S., stabilizers in frozen homogenized milk, 175; agitation while freezing homogenized milk, 812
- WISE, G. H., vitamin A and carotenoids of colostrum, 209; colostrum vitamin A and carotenoids, 458; soybean oils vs. butter oil for calves, 609; interference with activated glycerol dichlorohydrin test, 688; effect of carotenoid dispersion on absorption, 711*; milking procedures and mammary secretion composition, 719*
- WISEMAN, H. G., carotene in feed and vitamin A in butter, 714*; forage preservation procedures, 716*
- WRENN, T. R., testis biopsy of bulls, 327; hormone-induced mammary development, 707*
- WRIGHT, P. A., ascorbic acid in dried milk, 644; keeping quality of dried milk, 696*
- Y**ANG, S. P., effect of carotenoid dispersion on absorption, 711*
- YAPP, W. W., methods for estimating production, 621
- Z**ALETEL, J. H., unsaturation of milk fat, 700*
- ZEHNER, C. ERNEST, vaccination with *Brucella abortus* (strain 19), 914

SUBJECT INDEX OF ORIGINAL ARTICLES

ACETYLCHOLINE, effect on sperm O_2 consumption, 726*

Acidity,
effect on keeping quality of dried milk, 696*
titratable, electrometric measurements of, 699*

Activated glycerol dichlorohydrin, factors influencing use in vitamin A and carotenoid tests, 688

Aerobacter aerogenes, in bovine mastitis, 721*

Air cells, in ice cream, size measurements of, 696*

Alfalfa hay,
corn supplementation of, effect on milk production, 465

DDT content of, influence on DDT content of milk, 549

high and low fat concentrates with, 596

American Dairy Science Association,

44th annual meeting,

call for papers, 96

proceedings, 731

program, 477

registration and housing announcement, 381

officers and committees for 1949, 96

Amino acids,

effect of addition to Cheddar cheese, 769
in cheese, filter paper chromatography for, 705*

in colostrum and milk, 671

Androgens, fecal, excretion of by cows, 265

Ascorbic acid,

as stabilizer in frozen homogenized milk, 175

effect on,

keeping quality of dried milk, 644

oxidized flavor of frozen milk, 699*

oxidized flavor of milk, 306

in calf plasma and liver, influence of pasture and early rumen development on, 1016

in colostrum, prepartum milking and, 720*

in milk, oxidation, by preformed H_2O_2 , 163

in plasma, effect of *Brucella* vaccination on, 914

losses from lactose-enriched milk, 556

stability in water, 699*

Ash alkalinity, of dry buttermilk, 191

* Abstract of paper presented at annual meeting.

Aspergillus flavus, oxygen requirements of, 101

Aspergillus niger, oxygen requirements of, 101
Ayrshire females, type ratings as heifers and as cows, 375

BACTERIA, influence on semen fertility, 543

Bacteriophage,

action on *Streptococcus lactis*, 702*

multiplication of, effect of nutrition on, 701*

Banana meal, dehydrated, in calf rations, 361

Banana skins, nutrient composition of, 969

Blood plasma,

fat in, diurnal variations in, 709*

vitamin A and carotene in, effect of soybeans on, 565

Blood sugar, in ketosis, 718*

Bread,

dried buttermilk in, 703*

dry milk in, 703*

Brucella abortus, effect of home pasteurization on, 29

Brucella abortus (strain 19), vaccination with, effect on plasma vitamins, 914

Brucellosis control, Michigan program for, 729*

Buffer, carbonate-bicarbonate, for phosphatase test for cheese, 760

Bull fertility, effect of age and season on, 727*

Buttermilk, dry,

ash alkalinity of, 191

in bread, 703*

Butteroil,

fat fractions from, 700*

stale flavor component from, 701*

stale, solvent extraction from stale dried whole milk, 701*

Buying stations,

cream quality changes in, 935

cream segregation in, 534

CALCIUM,

in blood plasma, interrupted milking and, 977

in serum, at parturition of mastectomized cows, 927

Calf,

- losses in dairy herd, 712*
 mortality, in 2 Minnesota herds, 706*
 newborn, utilization of fetal vitamin A stores by, 764
- Calves,
 absorption of vitamin A and carotene by,
 effect of dispersion type on, 711*
 banana meal in rations of, 361
 carotene requirements for, 710*
 cotton seed oils in diets of, 665
 early rumen development in, influence on
 plasma composition and liver storage,
 710*
 health and performance of, effect of pre-
 partum vitamin A supplement on, 367
 heat tolerance of, 712*
 milk replacement for, 709*
 milk replacements for, 986
 milk substitutes for, 709*
 plasma levels of carotene and vitamin A in,
 influence of prepartum milking on, 711*
 plasma vitamin A and carotene of, effect of
 prepartum milking on, 1032
 soybean oil vs. milk fat for, 429
 soybeans in rations of, 609
 vitamin A and carotene depletion time of,
 418
- Candida lipolytica*, use in blue cheese from
 pasteurized milk, 993
- Carbohydrates, as energy sources for silage or-
 ganisms, 901
- Carotene,
 absorption, by calves, effect of dispersion on,
 711*
 absorption site in calves, 711*
 conversion to vitamin A, in calves, 711*
 depletion of calf stores of, 418
 determination in silage, 33
 in calf plasma, prepartum milking and, 1032
 in colostrum, 587
 in colostrum, effect of prepartum milking
 on, 919
 in feed, effect on vitamin A of butter, 714*
 in milk, interrupted milking and, 977
 in milk and plasma, soybean products and,
 565
 in plasma of calves from dams milked pre-
 partum, 711*
- Carotene requirements,
 for Guernsey reproduction, 714*
 of dairy calves, 710*
- Carotenoids,
 activated glycerol dichlorohydrin test for,
 in calf plasma and liver, influence of pasture
 and early rumen development on, 1016
 in colostrum,
 effect of ration supplements on, 458
 factors influencing, 209
 in milk, influence of pasture herbage on,
 955
 of milk fat, influence of temperature on,
 713*
- Catalase, oxygen damage to spermatozoa af-
 fected by, 353
- Cheese,
 blue,
 from pasteurized homogenized milk, chem-
 ical analysis and flavor score of, 1007
 use of *Candida lipolytica* cultures in,
 993
 use of mold-enzyme preparation in,
 1001
 manufacture from pasteurized milk, 704*
 surface growth and ripening of, 704*
 brick, yeast in surface smear of, 475
- Cheddar,
 amino acids in during ripening, 705*
 changes in diacetyl content of, 521
 diacetyl content of, 515
 effect of added amino acids on flavor of,
 769
 from pasteurized Canadian milk, 1
 radio-frequency pasteurization of, 790
 ripening temperature and tyramine con-
 tent and flavor of, 316
S. faecalis in, 630
 tyramine content and flavor of, 316
 tyramine content of, 630
- cottage, non-fat dry milk solids for manu-
 facture of, 705*
- free tryptophane in, 704*
 modified phosphatase test for, 751
 stirred curd, *S. faecalis* as culture for, 630
- Chromatography,
 filter paper, for amino acids in cheese, 705*
 separation of fatty acids by, 700*
- Clipping, cattle lice control by, 724*
- Cobalt tolerance, of young dairy cattle, 527
- Cod-liver oil,
 influence on milk and fat production, 133
 milk stability to oxidation affected by, 196
- Coliform bacteria,
 from mastitis, 721*
 soluble nitrogen produced in milk by, 652
 thermal death time studies on, 702*
- Colloidal protein particles, in skim milk, 697

Color, determination of, in dried milk products, 580

Colostrum,
 amino acid composition of, 671
 composition of, effect of prepartum milking on, 559, 919
 factors influencing carotene and vitamin A of, 209
 vitamin A, carotene and proximate composition of, 587
 vitamin content of, effect of prepartum milking on, 720*

Concentrates, high and low fat, fed with alfalfa hay, 596

Corn, as supplement to alfalfa hay, 465

Corn sugar, sweetness of, in ice cream, 697

Cottonseed oil, in calf diets, 665

Cow families, differences in production type, etc. of, 705*

Cow manure, male hormone in, 707*

Cows,
 dairy, time to eat rations, 716*
 mastectomized, blood composition at parturition, 927

Cream,
 churning time of, fat condition in cream and, 695*
 nordihydroguaiaretic acid as anti-oxidant in, 695*
 quality changes of, in buying stations, 935
 quality of, influence of segregation and mixing in buying stations on, 534
 stability of fat in, 679
 sweetened frozen, nordihydroguaiaretic acid as anti-oxidant in, 301

Cultured cream, body of, 695*

DAIRY cattle, heat tolerance of, 712*

Dairy program, integrated development of, 729*

D.H.I.A.,
 computing tables, 727*
 herd record book, suggested revisions for, 727*
 records, I.B.M. machines for processing of, 728*
 records, processing BDI 718 reports by machine, 728*

Diacetyl,
 in Cheddar cheese, 515
 in Cheddar cheese, changes during manufacture and curing, 521

Diluter, for bovine semen,

 composition effect on sperm motility, 345
 effect on livability and fertility, 604

Disease, infectious, as cause of infertility, 36

Diurnal variations, in blood plasma fat, 709*

Dried milk,
 in bread, optimum consumer preference for, 703*
 keeping quality of, effect of ascorbic acid addition to fluid milk on, 644

EMULSIFIERS, effect on physico-chemical properties of ice cream of, 696

Eosin-aniline blue stain, for semen quality, 723*

Equipment, dairy, sanitary standardization of, 701*

Escherichia freundii, in mastitis, 721*

Europe, western, dairy recovery in, 724*

FAT,
 in blood plasma, variations due to oil feeding, 709*
 in sow's milk, 22
 solidification of, in cream and butter, 695*
 stability of, in cream and butter, 679

Fat levels, in plasma, of mastectomized cows at parturition, 927

Fat production,
 influence of tocopherols and cod-liver oil on, 133
 methods of estimation, 621

Fatty acids,
 administration of, effect on blood ketone levels of ruminants, 817
 chromatographic separation of, 700*
 recovery by extraction and churning procedures, 435
 recovery by solvent extraction from acidified milk, 447

Feces,
 dry matter of, for calves on limited whole milk, 709*
 pH of, for calves on limited whole milk, 709*

Feed replacement value, of pasture, 717*

Fertility, of semen, bacterial numbers and, 543

Flavor, of Cheddar cheese, relation of diacetyl to, 515

Fluorine, in phosphate supplements for cattle, 228

4-H,

dairy project leaders, training of, 729*
 show programs, in Mississippi, 729*
 Furfural, in heated skim milk, 223

GASTRO-INTESTINAL motility, influence
 of lactose on, 139
 Gestation, prolonged, genetic origin of, 705*
 Growth, of cattle, vitamin E and, 495

HAY,
 barn-cured, nutrient conservation in, 716*
 dehydrated, nutrient conservation in, 712*
 feeding of, to cows on pasture, 717*
 palatability of, for calves, 10
 timothy, nutritive value at different maturity stages of, 659
 vitamin D in, 715*
 Health, of cattle, vitamin E and, 495
 Heart rates, factors influencing, 708*
 Heat tolerance,
 of calves of various breeds, 712*
 of dairy cattle, 712*
 Heifers,
 dairy, roughage and growth of, 715*
 growth of, wood molasses and, 719*
 Jersey, weight standards for, 718*
 Hemoglobin, influence of cobalt consumption
 on, 527
 Herd environment, estimating changes in, 706*
 High temperatures,
 effect on milk composition, 713*
 reactions of dairy cattle to, 713*
 Holstein-Friesian cattle,
 growth and reproduction of, 247
 inbreeding and outbreeding of, 247,
 Homogenization, measurement by turbidity,
 698*
 Hormone, male,
 in cow manure, 707*
 in cow manure, activation by temperature
 and drying, 796
 Hormones, for sterility treatment, 45
 Hortvet formula, for water added to milk,
 699*
 Hyaluronidase, in bull semen, turbidometric
 determination, 570
 Hydrogen peroxide,
 effect on oxidized flavor of frozen milk, 699*
 preformed, and ascorbic acid oxidation in
 milk, 163
 Hyperthyroidism, effect on milk production,
 707*

ICE cream,
 "cultured," manufacture of, 697*
 dehydrated whey in, 697*
 emulsifying agents in, 696*
 ice crystal and air cell size measurement in,
 696*
 shrinkage of, 697*
 sweetness of corn sugars in, 697*
 Ice crystals, in ice cream, size measurement of,
 696*
 Incidence of twinning, in 2 Minnesota herds,
 706*
 Inbreeding Holstein-Friesian cattle, effect on
 growth and reproduction, 247
 Infertility,
 approaching problem of, 92
 infectious disease as cause of, 36
 Inheritance, of functional reproductive inefficiency, 71
 Intestines, bovine, protozoa in, 806
 Iodobenzoate, for determination of reducing
 groups of milk and proteins, 698*

KEEPING quality, of pasteurized milk, in
 home refrigerators, 945
 Ketones, in bovine blood, fatty acid administration and, 817
 Ketosis,
 biochemical and histopathological studies of,
 718*
 blood sugar studies and, 718*

LACTASE, production of, lactose feeding
 and, 717*
 Lactose,
 alpha and beta, solubility method for determination of, 391
 effect on gastro-intestinal motility by, 139
 feeding, lactase production and, 717*
 milk enrichment with, effect on ascorbic
 acid losses, 556
 Leucocyte count,
 effect of *Brucella* vaccination on, 914
 of milk, effect on non-milking on, 649
 of postpartum milk, effect of prepartum
 milking on, 804
Leuconostoc citrovorum, penicillin inhibition
 of, 961
 Lice, cattle, clipping for control of, 724*
 Lipase, of milk,
 acid solvent extraction method for estimation of, 447

- measurement by solvent extraction and churning procedures for free fatty acids, 435
- Lyophilizing apparatus, 973**
- M**
- MAGNESIUM**, in serum, at parturition of mastectomized cows, 927
- Mammary development, hormone injections and, 707***
- Mammary edema, influence of feeding on, 721***
- Mammary secretions, composition of, effect of prepartum and postpartum milking on, 719***
- Manure, cow, male hormone activation in, 796**
- Mastitis,**
coliform bacteria from, 721*
diagnostic tests for, 720*
organisms causing, 720*
penicillin infusion for, 720*
therapeutic measures for, 720*
- Maturity stage, of timothy hay, effect on nutritive value, 659**
- Methylene blue test, for milk, variations in, 702***
- Milk,**
added water in, freezing-point determinations for, 699*
amino acid composition of, 671
ascorbic acid oxidation in, 163
composition of, high temperatures and, 713*
DDT content of,
dried,
 acidity variation and keeping quality of, 696*
 alpha and beta lactose determination in, 391
dried products of, color determination in, 580
dried whole,
 reducing systems of, 334
 stale flavor in, 383
evaporated,
 color of, 775
 effect of processing procedure on physical properties of, 775
 grain formation in, 775
 surface tension of, 775
frozen, effect of ascorbic acid and H_2O_2 on oxidized flavor in, 699*
frozen homogenized, effect of agitation during freezing on, 812
grading of, for manufactured dairy products, 703*
handling of, effect on bacteria and coliform counts of, 724*
heated,
 minor organic compounds from, 698*
 nitroprusside test applied to, 398
 sulfhydryl groups in, 398
homogenized, oxidized flavor in, effect of vitamin C elimination on, 786
homogenized frozen, stabilizers for, 174
nitrogen in, determination by direct nesslerization, 111
oxidized flavor of,
 effect of ascorbic acid on, 306
 influence of tocopherols and cod-liver oil on, 196
oxygen content of, 202
pasteurized,
 for Canadian Cheddar cheese, 1
 keeping quality in home refrigerators, 945
proximate composition of, interrupted milking and, 977
reducing groups in, *o*-iodosobenzoate for detmn. of, 698*
spray-dried, vitamin A stability in, 695*
surface tension of, changes in, 698*
turbidity measurement of homogenization efficiency in, 698*
vitamin A and carotene in, effect of soybeans on, 565
whole dried, solvent extraction of stale butter oil from, 701*
- Milk fat,**
saturation of, effect of pasture and external temperature on, 700*
vitamin A and carotene content of, influence of temperature on,
- Milk production,**
and delay after conditioned stimulus, 17
corn and alfalfa ration for, 465
excess concentrate feeding in Hawaii and, 725*
feeding hay to cows on pasture and, 717*
hyperthyroidism and, 707*
influence of tocopherols and cod liver oil on, 133
methods of estimation, 621
prepartum milking and, 725*
time between udder wash and attachment of milking machine, 637
vitamin E and, 495
- Milk replacements,**
for dairy calves, 986
in calf rations, 709*
- Milk substitutes, for dairy calves, 709***
- Milk surfaces, fresh, surface tension of, 406**

Milking, interruption of, influence on milk and blood serum, 977

Milking machine operation, extension education for, 728*

Molasses, wood, nutritive value of, 907

Molds, oxygen requirements of, 101

Mouse, male, influence of environmental temperature and thyroid status on, 712*

Mucus, bovine vaginal-cervical, pH *in vivo* and *in vitro*, 727*

NESSLERIZATION, for nitrogen in milk, 111

Nitrogen, in milk, determination by direct nesslerization, 111

Nitroprusside test, applied to heated milk, 398

Non-fat dry milk solids, for manufacture of cottage cheese, 705*

Non-milking, effect on leucocyte count of milk, 649

Nordihydroguaiaretic acid,

in cream, 695*

in cream and butter, 679

in sweetened frozen cream, 301

Nutrition, in treatment of bovine sterility, 60

OILS, feeding of, effect on fat in blood plasma, 709*

Oospora lactis, oxygen requirements of, 101

Outbreeding, of Holstein-Friesian cattle, effect on growth and reproduction, 247

Ovum, fertilized, recovery from living cow, 726*

Oxidized flavor,

in milk, effect of ascorbic acid on, 306

of milk, influence of tocopherols and cod liver oil on, 196

Oxygen, in milk, effect of handling and processing on, 202

Oxygen requirements, of 6 molds, 101

PARTURITION-ESTRUS interval, 726*

Pasture,

carotenoid content of, effect on carotene and vitamin A of milk, 955

effect on unsaturation of fat, 700*

influence on,

carotenoids, vitamin A and ascorbic acid of calves, 1016

rumen microorganisms of calves, 1025

Pasture forage, method for feed-replacement value of, 717*

Pasteurization,

home, effect on *Brucella abortus*, 29

of Cheddar cheese, by radio-frequency heat, 790

Penicillin,

as infusions for mastitis treatment, 720*

effect on lactic streptococci for cheese making, 961

for improving semen fertility, 722*

in semen dilutors, 722*

influence on bull semen fertility, 950

semen livability and bacterial content influenced by, 183

Penicillium expansum, oxygen requirements of, 101

Penicillium notatum, oxygen requirements of, 101

Penicillium roqueforti,

mold-enzyme preparation from, use in blue cheese, 1001

oxygen requirements of, 101

Phosphatase, rate of inactivation in milk, 509

Phosphatase test,

for cheese, carbonate-bicarbonate buffer for, 760

modification for cheese, 751

preservation of dairy products for, 166

Phosphorus,

in plasma, at parturition of mastectomized cows, 927

radioactive, distribution in cow's body, 717*

Plasma, vitamins in, effect of *Brucella* vaccination on, 914

Prepartum milking,

effect on,

calf plasma levels of carotene and vitamin A, 711*

colostrum composition, 559

colostrum composition, 919

leucocyte count in postpartum milk, 804

mammary secretion composition, 719*

performance of cows and calves, 719*

plasma vitamin A and carotene of calves, 1032

riboflavin and ascorbic acid content of colostrum, 720*

milk production following, 725*

Preservation, of dairy products, for phosphatase test, 166

Production, of milk and fat, influence of tocopherols and cod liver oil on, 133

Production records,

analysis of, in artificial insemination program, 730*

computation of, 728*
 Proteins, plant and animal, semen production and fertility of bulls fed, 292
 Protozoa, occurrence in bovine stomach, 806
 Proved sons, for evaluation of transmitting ability of sire, 706*

QUATERNARY ammonium compounds, effect of metallic ions on, 703*

RED cell volume, influence of cobalt consumption on, 533
 Reducing groups, in proteins and milk, 698*
 Reducing systems, of dry whole milk, 334
 Reproduction,
 carotene requirements for, 714*
 vitamin E and, 495
 Reproductive failures, in cows of low fertility, 237
 Reproductive inefficiency, inheritance of functional causes of, 71
 Resazurin test, for milk, variations in, 702*
 Review,
 hormones for treatment of sterility, 45
 infectious disease as cause of infertility, 36
 inheritance of functional causes of reproductive inefficiency, 71
 lactose and gastro-intestinal motility, 139
 nutrition and bovine sterility, 60
 Riboflavin, in colostrum, prepartum milking and, 720*
 Roughage, and growth of dairy heifers, 715*
 Roughages, vitamin D content of, 715*
 Rumen,
 digestion and synthesis in, effect of saliva on, 123
 early development of, influence on carotenoids, vitamin A and ascorbic acid of calves, 1016
 establishment of microorganisms in, effect of pasture and rumen inoculation on, 710*, 1025
 inoculation of, for calves, 1025

SALIVA, bovine, chemical and physical properties of, 123
 Sanitary standardization, of dairy equipment, 701*
 Semen,
 bovine,
 effect of testis biopsy on, 327

livability and bacterial content of, effect of penicillin and streptomycin on, 183
 thyroxine and fertility of, 322
 triphenyltetrazolium chloride for vital studies of, 1013
 cooling of, citrate-sulfanilamide-yolk extender and, 723*
 dilution of,
 buffered whole egg for, 722*
 on basis of numbers of spermatozoa, 721*
 penicillin and sulfanilamide in, 722*
 to higher levels, 722*
 fertility of,
 effect of penicillin and sulfanilamide on, 722*
 effect of penicillin on, 950
 improvement by penicillin, streptomycin and sulfanilamide, 722*
 influence of bacterial numbers on, 543
 frequency of collection of, 723*
 oxygen consumption of,
 effect of thyroxine and acetylcholine on, 726*
 testosterone effect on, 725*
 production, influence of plant and animal proteins on, 292
 quality of, cosin-aniline blue stain for, 723*
 turbidometric hyaluronidase determination in, 570
 Sex ratio, in 2 Minnesota herds, 706*
 Sherbets,
 dehydrated whey in, 697*
 whey use in, 696*
 Shrinkage, of ice cream, 697*
 Silage,
 carotene determination in, 33
 wilted, nutrient conservation in, 716*
 Silage organisms, energy sources for, 901
 Sire selection, a different slant on, 730*
 Skim milk, heated, isolation of furfural from, 222
 Sodium citrate, as stabilizer for frozen homogenized milk, 175
 Soil fertility, effect on dairy cows, 716*
 Soluble nitrogen in milk, influence on *S. lactis* and coliform bacteria on, 652
 Sow's milk, fat and vitamin A in, 22
 Soybean oil,
 crude expeller, calf feeding with, 429
 hydrogenated, calf feeding with, 429
 Soybean oil meal, effect on plasma and milk vitamin A and carotene, 565
 Soybeans,

and vitamin A requirements of dairy cattle, 714*
 in calf rations, 609
 raw, effect on plasma and milk vitamin A and carotene concentrations, 565
Spermatozoa,
 livability and fertility of, effect of diluters on, 604
 motility of, effect of diluter composition on, 345
 oxygen damage to, effect of catalase on, 353
Stabilizers, for frozen homogenized milk, 175
Stale flavor,
 of butter oil, 715*
 of dried whole milk, 383
Sterility,
 hormone treatment for, 45
 nutrition and treatment of, 60
Stilbestrol, in bovine mammary development, 707*
Stimulus, conditioned, milk production after, 17
Stomach, bovine, protozoa in, 806
Streptococcus agalactiae, in mastitis, 720*
Streptococcus cremoris,
 bacteriophage multiplication on, 701*
 effect of penicillin and other antibiotics on, 961
 nutrition of, 701*
Streptococcus dysgalactiae, in mastitis, 720*
Streptococcus faecalis, in Cheddar cheese, 630
Streptococcus lactis,
 action of bacteriophage on, 702*
 bacteriophage multiplication on, 701*
 effect of penicillin and other antibiotics on, 961
 nutrition of, 701*
 soluble nitrogen produced in milk by, 652
Streptococcus uberis, in mastitis, 720*
Streptomycin,
 for improving semen fertility, 722*
 semen livability and bacterial content influenced by, 183
Subtilin, effect on lactic streptococci for cheesemaking, 961
Sulfanilamide,
 for improving semen fertility, 722*
 in semen diluters, 722*
Sulphydryl groups, in heated milk, 398
Sulfonamides, in semen diluters, 604
Surface growth, on blue cheese, 704*
Surface tension,
 of fresh milk surfaces, 406
 of milk, 698*

Sweet potatoes, dehydrated, as feed for lactating cows, 725*
 Sweetness, of corn sugar products in ice cream, 697

TEMPERATURE,

 effect on carotene and vitamin A of milk fat, 718*
 external, effect on fat unsaturation, 700*
 Testis biopsy, semen influenced by, 327
Testosterone, *in vitro* effect on O₂ consumption of sperm, 726*
Thermal death time studies, on coliform bacteria, 702*
Thyroid gland, size in southern cows, 708*
Thyroprotein,
 feeding during lactation decline, 708*
 feeding during first lactation, 278
 low-level feeding for 52 weeks, 257
Thyroxine,
 effect on fertility of semen, 322
 effect on sperm O₂ consumption, 726*
Tocopherols,
 in milk, effects of feeding on, 700*
 in ration, effect on colostrum, 458
 influence on milk and fat production, 183
 milk stability to oxidation affected by, 196
Transmitting ability, of sire, number of proved sons necessary for evaluation of, 706*
Tricalcium phosphate, fused, as low-fluorine phosphorus supplement, 228
Triphenyltetrazolium chloride, vital studies on semen with, 1013
Tryptophane, free, determination in cheese, 704*
Type ratings, of Ayrshires, as heifers and as cows, 375
Tyramine,
 in Cheddar cheese, 316, 630

UDDER treatment, milk let-down influenced by, 725

VISCOSITY,

 measurement of, torsion pendulum viscometers for, 452
 of milk products, 452
Vitamin A,
 absorption,
 by calves, effect of dispersion on, 711*
 by ruminants, 714*

activated glycerol dichlorohydrin test for, 688
depletion of calf stores of, 418
effect of prepartum milking on, 919
fetal stores of, utilization by newborn calf, 764
in butter, carotene in feed and, 714*
in calf plasma, prepartum milking and, 1032
in calf plasma and liver, influence of pasture and early rumen development on, 1016
in colostrum, 587
 effect of ration supplements on, 458
 factors influencing, 209
in milk,
 influence of pasture herbage on, 955
 interrupted milking and, 977
in milk and plasma, soybean products and, 565
in plasma,
 effect of *Brucella* vaccination on, 914
 of calves from dams milked prepartum, 711*
in sow's milk, 22
in spray-dried milk, stability to drying, 695
of milk fat, influence of temperature on, 713*
prepartum supplement of, effect on calves, 367

requirements by cattle, relation of soybeans to, 714*
supplementing calf rations with, 418
Vitamin A alcohol, in ration, effect on colostrum, 458
Vitamin A ester, in ration, effect on colostrum, 458
Vitamin B₁₂, role and sources of mammals, 715*
Vitamin C, elimination of, effect on oxidized flavor of homogenized milk, 786
Vitamin D, in roughages, 715*
Vitamin E, effect on reproduction, health, milk production and growth, 495
Vitamins, fat-soluble, stability in cream and butter, factors influencing, 679

W EIGHT standards, for Jersey heifers, 718*

Whey,
 dehydrated, in ice cream and sherbets, 697*
 use in sherbets, 696*

Wood molasses, and growth of heifers, 719*

Y EAST, in surface smear of brick cheese, 475

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

P. E. ELLSWORTH, Acting Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

F. E. NELSON, Editor
Ames, Iowa

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM
MANUFACTURERS

R. C. HIBBEN, Executive Secretary
1105 Barr Bldg., Washington, D. C.

MILK INDUSTRY FOUNDATION
B. F. CASTLE, Executive Director
1001 Fifteenth Street, N.W., Washington 5. D. C.

Editorial Committee

H. H. SOMMER, Madison,
Wisconsin, A. D. S. A.

HAROLD PRATT, Philadelphia,
Pennsylvania, I. A. I. C. M.

G. D. TURNBOW, Oakland
California, I. A. I. C. M.

A. J. POWERS, Brooklyn,
New York, M. I. F.

V. CHRISTIANSEN, Chicago,
Illinois, M. I. F.

CONTENTS

Animal Diseases
Butter
Condensed and Dried Milks;
By-products
Cheese
Dairy Bacteriology
Dairy Chemistry

Dairy Engineering
Dairy Plant Management
and Economics
Feeds and Feeding
Genetics and Breeding
Herd Management
Ice Cream

Milk
Milk Secretion
Nutritive Value of Dairy
Products
Physiology and Endo-
crinology
Sanitation and Cleansing

ABSTRACTS OF LITERATURE

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

1. **Penicillin therapy in chronic bovine mastitis.**
II. **Penicillin levels in the udder during treatment.** R. A. PACKER, Iowa State College, Ames. *Am. J. Vet. Research*, 9, 32: 259-263. July, 1948.

Penicillin in saline solution was injected into udders. The data indicated that less penicillin at 12 hr. after injection had left the quarters producing the greater quantities of milk. Differences were minor after a period of 24 hr. There were no marked differences between the quantities of penicillin which remained in the udder secretions 24 hr. after injection when 25,000 and 200,000 units were used. Similar levels were obtained 12 hr. post-injection in secretions from a staphylococcal infected quarter and a non-infected one. Levels of penicillin in udder secretions were fairly similar regardless of the time during milking when the samples were collected. Frequency of injection influenced the levels maintained in udder secretions more than the quantities injected.

W. D. Pounden

2. **Penicillin therapy in chronic bovine mastitis.**
III. **Treatment of mastitis.** R. A. PACKER, Iowa State College, Ames. *Am. J. Vet. Research*, 9, 32: 264-269. July, 1948.

Little difference in results was obtained in the treatment of streptococcal udder infections between 1 or 2 injections of penicillin at 24 hr. intervals and 3 or 4 injections at 12 hr. intervals. Average results were 83% for all cases using 25,000 to 200,000 units in 50.0 cc. saline solution. Successfully treated staphylococcal cases were 22% for 1 or 2 injections and 48% for 3 or 4 injections.

W. D. Pounden

BUTTER

O. F. HUNZIKER, SECTION EDITOR

3. **Apparatus for the molding of butter and like products.** J. O'CONNELL. U. S. Patent 2,451,301. 20 claims. Oct. 12, 1948. *Official Gaz. U. S. Pat. Office*, 615(2): 459. 1948.

A machine is described which continuously and automatically molds butter into various shaped and sized pieces. A number of identical molds rotate from beneath the filler to a member which

discharges the product from the mold thence returning to the filling position again.

R. Whitaker

Also see abs. no. 21.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

4. **Quality improvement in Canadian Cheddar cheese.** W. H. SPROULE. *Can. Dairy Ice Cream J.*, 27, 7: 29-32. July, 1948.

The concentration of effort to improve Cheddar cheese should include (a) explicit information to the producer of sanitary methods of production and cooling of milk; (b) more extensive use of the single service milk filter on the farm; (c) checking of milk on the receiving platform, aided by reductase test taken at regular intervals; (d) adoption of an organized plan of sediment testing of milk and cheese; (e) more extensive use of filter strainers at the factory and (f) prevention of coal dust or about material getting into milk and curd during the manufacturing process.

H. Pynson

5. **The manufacture of low-acid rennet-type cottage cheese.** H. R. LOCHRY, Bureau of Dairy Ind., Agr. Research Admin. *Southern Dairy Products J.*, 44, 3: 84-85, 88-90, 92, 94. Sept., 1948.

Low-acid, rennet-type cottage cheese is a superior product. It may be manufactured by the following procedure: Set at 70 to 72° F. skim milk pasteurized by the holding method or the short-time method and add enough active starter (about 0.5 to 1%) to produce from 0.51 to 0.53% acidity in the whey by the time curd is to be cut. Add 1 ml. of rennet per 1,000 lb. of skim milk. Maintain uniform temperature. Cut the curd into half-inch cubes. Cover the curd with at least 2 inches of water at 115 to 120° F. and apply steam in the jacket of the vat to raise the temperature in 1 to 2 hr. to 110 to 120° F., or until the curd has the proper firmness when tested after dipping a sample in cold water. Stir the curd carefully to avoid breaking. Wash the curd with tap water and then with ice water. Drain for 1 hr. and add 1 lb. of salt per 1,000 lb. of skim milk. Hold in cooler at 32 to 40° F. for 4 hr. or until day of sale.

To each 100 lb. of curd add 1 lb. of salt, 30.5 lb. of pasteurized 18% cream, preferably homogenized.

enized, and enough 4.2% milk to produce the desired consistency. F. W. Bennett

6. New product developments leading to greater cheese sales. G. H. WILSTER. *Can. Dairy Ice Cream J.*, 27, 6: 52-62. June, 1948.

The factors involved in the expansion of cheese sales are: (a) continued high consumer income and the desire by the consumer for good food of which cheese is one; (b) shortage of meat and meat products; (c) varieties of cheese other than Cheddar should be offered for sale; (d) cheese quality improvement and standardization of quality; (e) reduction in cost of production through developing economics in the manufacture and packaging of cheese and more efficient utilization of whey; (f) improved merchandising methods; (g) further education of consumer regarding the nutritional value of cheese and in the methods of using cheese to advantage in preparing menus; and (h) education of homemakers in methods of serving and caring for cheese in the home so that the product will have maximum palatability.

H. Pyenson

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

7. A modification of the Voges-Proskauer test for rapid colorimetric determination of acetyl-methylcarbinol plus diacetyl in the butter cultures. N. KING. *Dairy Inds.*, 8, 9: 860-861. Sept., 1948.

A modification of the Ritter test for acetyl-methylcarbinol plus diacetyl is described. Reagent A is a 30% aqueous solution of KOH. Reagent B contains 0.1 g. dicyandiamide and 4.0 g. α -naphthol in 10.0 ml. amyl alcohol and 50.0 ml. ethyl alcohol.

The test is performed as follows: To 2 ml. of the untreated culture are added 1 ml. of reagent A and 1 ml. of reagent B. The reaction is carried out in a wide test tube to obtain better contact with the air, or strictly speaking with the oxygen of the air, which may be regarded as the third reagent. The mixture is brought to about 30° C. in a water bath, held 30 minutes and repeatedly shaken. A more or less intense red-lilac coloration develops, depending on the content of acetylmethylcarbinol. The fluid then is filtered through a paper filter. The color intensity of the clear filtrate is compared with the intensity of a color standard which corresponds to a content of acetylmethylcarbinol of 2 mg. per 100 ml. of cultures. A dilution technic is described to aid in quantitative measurements. The composition and preparation of the color standard is described in detail. G. H. Watrous, Jr.

8. Grading milk with the resazurin test. N. S. GOLDING. *Can. Dairy Ice Cream J.*, 27, 7: 33, 82. July, 1948.

One of the official methods of grading milk in the 9th edition of *Standard Methods for Dairy Products* of the American Public Health Association will be the resazurin test. Prepared sterile vials have been made available by the Dairy Husbandry Department at the State College of Washington. The teaching of the resazurin test by a practical short course invites many questions and probably is the best way for field men and others to understand the work.

H. Pyenson

9. Direct versus plate counts. L. D. SEARING. *Can. Dairy Ice Cream J.*, 27, 7: 37-39. July, 1948.

The usual advantages and disadvantages of the direct microscopic count and the plate method of counting bacteria in milk are outlined. Some practical comparisons are given. On 129 samples, the average plate count was 20% higher than the average direct count. H. Pyenson

10. A combination of the resazurin test and the direct microscopic count for the bacteriological examination of milk. A. L. BORTT and R. D. SPENCER. *Mich. Agr. Expt. Sta., East Lansing. J. Milk and Food Technol.*, 11, 5: 256-258, 268. Sept.-Oct., 1948.

A direct comparison of the resazurin test and direct microscopic count is not possible in checking the quality of milk. However, the authors suggest a combination of the resazurin test and direct microscopic count in checking the milk. All samples placed in class 1 by the reduction test in general showed a low bacterial count, while those samples placed in class 4 definitely were of low quality.

Apparently, the resazurin test could be used as a screening test to eliminate high quality milk samples from further examination. The test also may be useful in detecting low quality milk subject to further examination and field work. H. H. Weiser

11. Significance of coliform bacteria in pasteurized milk and milk products. M. P. BAKER. *Dept. of Dairy Ind., Iowa State College, Ames. Milk Plant Monthly*, 37, 8: 82-84, 95. 1948.

Coliform organisms consistently come into pasteurizing plants with the raw milk, and while they do not resist pasteurization, except rarely, there are a number of ways they can find their way around the pasteurizing process and into the pasteurized product. Their presence in the pasteurized product indicates that they got in

there after heating, which means unclean equipment or improper protection of the product against drip contamination, hand contact, etc. Their numbers in pasteurized products depend upon the amount of contamination and also on the amount of the growth that may have taken place in the product. Coliform count thus is an index to the sanitation in the plant, particularly in the handling after pasteurization.

G. M. Trout

12. The potentiating action of sulfonamides on the *Brucella* antibody-complement system. I. F. HUDDLESON, Mich. State College, East Lansing. Am. J. Vet. Research, 9, 32: 277-285. July, 1948.

Previous work by the same author had shown that undiluted fresh serum or plasma from most normal as well as immune cattle contained specific antibodies which in combination with complement killed *Brucella* organisms. However, a similar effect could not be demonstrated in similar blood constituents from *Brucella*-infected cattle. Under certain conditions when complement was added to the latter serum or plasma, growth *in vitro* of the organisms was delayed. Further work showed that the temporary growth-inhibiting effect of sodium sulfonamide salts for *Brucella* organisms in suitable culture medium was enhanced to a bactericidal effect when fresh normal serum was added, but not when serum from infected animals was used. The failure of the latter serum is explained as being due to the prozone antibody phenomenon.

Experimentally produced brucellosis in guinea pigs was markedly alleviated by the feeding of 0.2 g. sulfadiazine, along with the intraperitoneal injection of normal or immune rabbit serum.

W. D. Pounden

Also see abs. no. 1, 2, 69, 70.

DAIRY CHEMISTRY

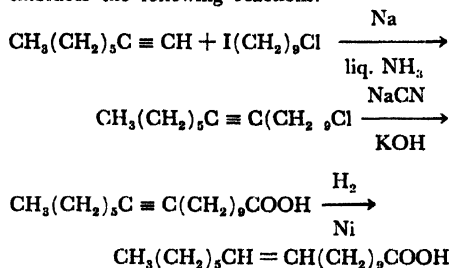
H. H. SOMMER, SECTION EDITOR

13. A synthesis of *cis*-11-octadecenoic and *trans*-11-octadecenoic (vaccenic) acids. K. AHMAD, F. M. BUMPUS, AND F. M. STRONG, Dept. of Biochem., Univ. of Wis., Madison. J. Am. Chem. Soc., 70, 10: 3391-3394. Oct., 1948.

The configuration of natural vaccenic acid (isolated from beef tallow) is *trans*-11-octadecenoic acid. Synthesis of the acid was accomplished readily with excellent yields by condensation of 1-octyne with 1-chloro-9-iodonane and conversion of the resulting 1-chloro-10-heptadecyne, via the nitrile, to 11-octadecynoic acid. Partial hydrogenation of the acetylenic bond

in the latter produced only *cis*-11-octadecenoic acid which was isomerized to the *trans* form (vaccenic acid) with selenium.

Synthetic vaccenic acid was found to melt at 43-44° C. Natural vaccenic acid melts at 39° C., according to Bertram. The *cis* isomer melts at 10.5-12° C. The method of synthesis embraces the following reactions:



H. J. Peppler

14. A comparison of semi-monthly and monthly composite samples as a basis of paying for milk. E. O. HERREID, Univ. of Ill., Urbana. Milk Dealer, 37, 10: 58-64. July, 1948.

Data are presented comparing fat test of semi-monthly and monthly composite samples. If preserved composite milk samples are kept in clean, sterile bottles at 50° F., or less, in good physical condition and free from mold growth and are properly prepared, sampled and tested by the Babcock procedure, semi-monthly composites yield slightly more milk fat than monthly composites. However, the difference is so small that testing can be done monthly without material injustice to the patron or to the plant. Semi-monthly and monthly composites can be reprepared and retested once, after holding for 48 hrs., and the results will be reasonably comparable with those secured from tests made at the end of the sampling period. Significantly lower results are apt to be obtained if composite samples are reprepared and retested a second or a third time.

C. J. Babcock

15. A procedure for determination of DDT in milk. D. E. H. FREAR, Dept. of Agr. and Biol. Chem., Penn. State College. Milk Plant Monthly, 37, 8: 96-99. Aug., 1948; Milk Dealer, 37, 12: 58-62. Sept., 1948.

The author describes in specific detail the method of determining quantitatively the amount of DDT present in milk.

G. M. Trout

16. Manufacture of artificial textile fibers. A. FERRETTI. (vested in Attorney General of U. S.)

U. S. Patent 2,450,889. 2 claims. Oct. 12, 1948. Official Gaz. U. S. Pat. Office, 615(2): 357. 1948.

A textile fiber is produced by dissolving casein in a mixture of caustic alkali and Na and K silicates, spinning fibers from the mixture and then coagulating same with an acid.

R. Whitaker

Also see abs. no. 7, 40, 41.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

17. **Color combinations—their importance to efficient, sanitary dairy products plants.** A. C. TERRELL, A. C. Terrell Co., St. Louis, Mo. Southern Dairy Products J., 44, 4: 40, 90-91, 98. Oct., 1948.

Paints for dairy plants should be selected to protect buildings and equipment and to safeguard the employees and add to their efficiency. Gloss enamel finishes last longer than flat paints, are washable and have greater reflection. Enamels should be selected for resistance to moisture, steam, oils, fats, acids and alkalies, to which they may be exposed. Colors which properly reflect or absorb light, have warmth or coolness, stimulate or relax to suit the location should be selected. Light gray is recommended for equipment to increase visibility. Color standards for safety should be observed, especially to call attention to hazards

F. W. Bennett

18. **Heating apparatus.** D. D. PEBBLES. (assigned to Golden State Co.) U. S. Patent 2,452,260. 3 claims. Oct. 26, 1948. Official Gaz. U. S. Pat. Office, 615(4): 988. 1948.

A heater for liquids, including food products like milk, is constructed of a tube into one end of which steam is admitted. The fluid to be heated is introduced directly into the flowing steam through a series of equally spaced tangentially directed jets which cause the liquid to spiral inwardly and to be discharged through the end of the tube opposite to the steam inlet.

R. Whitaker

19. **Centrifugal separator.** B. S. HUGHES. (assigned to Zarembo Co.) U. S. Patent 2,452,465. 1 claim. Oct. 26, 1948. Official Gaz. U. S. Pat. Office, 615(4): 1038. 1948.

An entrainment separator for vacuum pans is described.

R. Whitaker

20. **Filter.** J. P. O'MEARA. (assigned to W. M. Sprinkman Corp.) U. S. Patent 2,452,486.

4 claims. Oct. 26, 1948. Official Gaz. U. S. Pat. Office, 615(4): 1044. 1948.

A filter for fluids, such as water and milk, consisting of a series of horizontal disks, perforated and so arranged that the product passes slowly upward through pads of cloth, cotton, paper, etc., is described. Heavy particles collect on the plates rather than the pads.

R. Whitaker

21. **Churn.** W. RIVERS. U. S. Patent 2,452,492. 1 claim. Oct. 26, 1948. Official Gaz. U. S. Pat. Office, 615(4): 1045. 1948.

A small vertical churn having an oscillating agitator supported on the bottom is described.

R. Whitaker

22. **Dairy plants need pure, fresh air.** CLYDE TOMPKINS, Reynolds Electric Co., Chicago, Ill. Milk Dealer, 37, 10: 68-70. July, 1948.

The need for pure, fresh air in dairy plants is stressed. Enclosed or partially enclosed rooms can secure forced air circulation by: (a) air conditioning, whereby washed or filtered air of a desired temperature is forced into the room through ducts by blower type fans; (b) an exhaust fan, whereby a fan is located in an outer wall, usually near the ceiling of a room and there normally is an air intake near the floor of the room; (c) the use of free circulating fans which keep the air in the room in motion.

The types of free air circulating fans are: (a) the horizontal blowing fan which keeps the air in motion to a greater or less degree but leaves dead air spots in the room; (b) the horizontal, oscillating fan which blows a while in one direction and then automatically changes and blows it in another direction, the various currents set up by this action tending to neutralize each other and leave much of the air in the room stagnant; (c) the fan which blows upwards at high velocity, the air velocity, on striking the ceiling being transformed into an expanding turbulence which travels slowly along the ceiling, down the walls and up to the fan again. Tests have shown the latter type to be most effective.

The importance of air circulation in a refrigerated room also is discussed.

C. J. Babcock

Also see abs. no. 3.

DAIRY PLANT MANAGEMENT AND ECONOMICS

23. **The need for standardization of operating methods in dairy processing plants.** W. H. E.

REID, Univ of Mo, Columbia Southern Dairy Products J, 44, 4 45, 84-85, 87-88 Oct, 1948

The following points have been considered by the most successful dairy plant operators in standardizing their operating methods good will of consuming public based upon quality of product, efficiency of operation, feeling of personal responsibility and importance of all employees, character and integrity of employees, ability of employees to command respect and cooperation of other people, cooperation with health and control officials, convenience and sanitation of rest rooms, locker rooms and shower baths, air conditioning of plant, and good housekeeping to maintain the plant and equipment sanitary, attractive and in most efficient working order

I W Bennett

24. What is any business worth? A C KIRCHLIN, Public Accountant, Pompton Lakes, N J Ice Cream Rev, 31, 3 52, 54, 56 Oct, 1948

The tangible net worth of the business may be arrived at quite readily by deducting from the current market value of the physical assets the value of all liabilities. The difference between these 2 values represent the current tangible net worth of the business

The intangible value of the business is made up of good will and earning power. One method of arriving at a fair value for good will is to determine the average net profit, the average capital investment and the average return on the capital investment at 5% for the past 3 years of operation. Then subtract from the average net profit the average return on capital investment figured at 5%. Multiply the difference by 5, representing the "number of years purchase"

The figure representing the number of years purchase may vary depending upon the length of time the business has been in operation, the trend in profits for the business and general business conditions. Usually a figure between 2 and 10 is used as the number of years purchase. The value of the business as determined by this procedure, would be the sum of the tangible net worth plus the value arrived at for good will.

Another procedure for arriving at the value of good will is to multiply the average earnings for the business during the past 5 years by 10 and deduct from this figure the value of the assets. The difference between these 2 values represents the value of good will.

Anyone who is contemplating the sale of his business would do well to figure the value of good will by each method presented before determining the value to be placed on the business

W J Caulfield

25. Co-operatively built plant ANONYMOUS Milk Dealer, 37, 9 50 51, 71 June, 1948

Fourteen independent milk dealers in Springfield, Mass pooled resources to construct a jointly operated \$350,000 pasteurizing plant. Carrying on processing operations in the new plant under the name of Dairy Center, Inc., the 14 individual milk dealer owners are continuing to distribute co-operatively processed dairy products independently under their own firm names in their own communities C J Babcock

26. Where do we go from here? A R STEVENS, National Dairy Products Corp, New York City Milk Dealer, 37, 9 168-174 June, 1948

A discussion of the opportunities in the dairy industry and the factors that challenge these opportunities is presented. The following are mentioned as some of the things that should be included in the programs of all parts of the dairy industry (a) thorough and consistent dissemination of information regarding the economics of the dairy business (b) a fair and reasonable profit without tampering or interfering with the quality of dairy products (c) educate dealers to retail dairy products to the public at fair mark ups (d) make dairy products convenient to the public to buy, which means distributing them through every possible outlet, (e) surround the sale and consumption of dairy products with an atmosphere of cleanliness and sanitation, and (f) in addition to nutrition, give greater emphasis to the goodness and appetite appeal of dairy products

C J Babcock

27. Cutting operating cost through better management. E L BABUM Iowa State College, Ames Milk Dealer, 37, 12 53, 75 Sept, 1948

The only recourse left to the dairy plant operator in meeting the increased cost of his fluid milk supply, as well as the increased cost of equipment, labor, etc., is to cut costs through better management. To do this, it is necessary to (a) keep closer records of all milk utilization and butterfat losses, container costs, etc (b) keep daily plant records in order to locate excess plant labor and to show where it can be shifted (c) study plant operations to observe labor inefficiencies and inefficiencies caused by improper placement of equipment (d) use straight-line methods of machinery depreciation

C J Babcock

Also see abs no 42, 48, 51, 52, 53, 54

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

28. Seasonal variations of carotene and other nutritionally important constituents in the two pasture grasses *Dallis* and *Carpet*. J. R. KUPPERS, L. L. RUSOFF, AND D. M. SEATH. J. Agr. Research, 77, 2: 55-63. July 15, 1948.

A study was made of the seasonal trends of some nutritionally important constituents of 2 species of pasture grasses, *Carpet* (*Axonopus affinis*) and *Dallis* (*Paspalum dilatatum*) during a growing season of 9 months. On the average and over the greater part of the season, *Dallis* grass had a higher content of crude carotene, crude protein, lignin, cellulose, calcium, phosphorus, ash and moisture. During the hot months of Aug. and Sept., *Carpet* grass was as good as, or better than, *Dallis* grass as a source of carotene, protein, calcium and phosphorus. Seasonal variations in composition were less for *Carpet* grass than for *Dallis* grass. Significant correlations were found between crude carotene and crude protein in both *Carpet* grass (+0.52) and *Dallis* grass (+0.73). H. Pyenson

29. Feed all the colostrum. N. N. ALLEN, Univ. of Vt., Burlington. Hoard's Dairyman, 93, 21: 805. Nov. 10, 1948.

Three groups of calves were used to determine to what extent stored colostrum could replace marketable milk for calf raising. Group I (control group) was fed the dam's milk for 10 d. and then changed to mixed milk. Group II was fed fresh colostrum the first d. The remainder of the colostrum produced through the third d. after parturition was frozen and fed during the first 10 d. of the calf's life in the order that it was produced. Then the calves were put on mixed milk. Groups I and II were fed skim milk from 5 to 9 weeks of age. Group III was handled like the second for the first 10 d., after which they were fed stored colostrum until 9 weeks of age. The all-colostrum calves (group III), which had averaged 5.5 lb. lighter at birth, averaged 3 lb. heavier at 9 weeks of age. Group II, which averaged 0.8 lb. lighter than the check lot at birth, averaged almost 2 lb. heavier at 9 weeks. While the calves of all 3 groups were thrifty and husky, the all-colostrum animals had the sleekness typical of calves fed whole milk. Colostrum can replace an equal amount of salable milk when used properly. J. B. Frye, Jr.

Also see abs. no. 68.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

30. Hyaluronidase activity of spermatozoa. O. HECHTER, AND Z. HADDIAN, Worcester Foundation for Experimental Biology and Tufts Medical School. Endocrinology, 41, 2: 204. Aug., 1947.

Rabbit semen, collected with an artificial vagina, was utilized and in all instances the semen was studied within 24 hr., and in most instances within 1 hr., after collection. The semen sample was centrifuged, and successive saline washings made of the spermatozoa followed by centrifugation. Hyaluronidase activity was measured by either a viscometric or a skin spreading method of assay. Using the spreading method of assay, it consistently was observed that the hyaluronidase concentration in the second, third, fourth, fifth, and sixth washings remained nearly equivalent to the original seminal plasma. In the seventh washing, the enzyme activity fell off at least 10-fold. It was concluded that the hyaluronidase activity obtained in the washings of sperm either must be derived from the spermatozoa or closely associated with them. Ralph P. Reece

31. Motility of bovine spermatozoa. C. K. RAO AND G. H. HART, Univ. of Calif. Am. J. Vet. Research, 9, 32: 286-290. July, 1948.

Motility of sperm normally is initiated in the ampullae but can be induced in sperm from deeper regions of the reproductive tract. It is the outcome of admixture of the sperm with accessory secretions rich in electrolytes and the influence of a series of functions of the organs of the reproductive tract. Examination and evaluation of bovine semen as regards motility are discussed in detail. W. D. Pounden

Also see abs. no. 67.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

32. Teat draining tube. H. A. ALCORN. U. S. Patent 2,450,217. 1 claim. Sept. 28, 1948. Official Gaz. U. S. Pat. Office, 614(4): 982. 1948.

A tube having one end tapered to a blunt point for inserting into a cow's teat to facilitate drainage of milk is described. Spiral channels on the surface permit the milk to flow between the teat wall and the device proper. R. Whitaker

33. Escharotic solution for dehorning calves. J. E. GUTHRIE. (assigned to Hess and Clark, Inc.) U. S. Patent 2,450,953. 2 claims. Oct.

12, 1948 Official Gaz U S Pat Office, 615
(2) 372. 1948

Calves may be dehorned by antimony chloride dissolved in nitrocellulose lacquer

R Whitaker

34. Cutting production costs. ANONYMOUS
Milk Dealer, 37, 12 48-49, 100-101 Sept,
1948

The experiments being conducted on an all-electric dairy farm by the Univ of Wis College of Agriculture in cooperation with the Wis Utilities Assoc to determine means of reducing labor on dairy farms are described Principal equipment are a barn cleaner, silo unloader, silage dispensing cart, cow trainers, ventilating fan, hay drier, mechanical milk cooler, milking machine, hot water heater, can rack and sink for washing hands The silo unloader and barn cleaner are expected to be the greatest labor and time savers The use of a flow diagram to check routes of feed distribution, movement of bedding, carrying of milk from cows to milk house and other farm chores is suggested as another means of cutting labor costs C J Babcock

Also see abs no 29

ICE CREAM

C D DAHLE, SECTION EDITOR

35. Chocolate coated ice cream. J R DEVOR
Can Dairy Ice Cream J, 27, 6 34-36 June,
1948

Chocolate coated ice cream bars are not advertised or displayed as much as other ice cream products or chocolate candies The high cost of chocolate can be overcome by making a smaller bar, putting the chocolate on thinner and raising the price of the bar In Canada there is a lack of trained chocolate temperers for the ice cream industry Research work on chocolate has developed a satisfactory coating for ice cream Ideal storage for chocolate is between 50 and 60° F The chocolate should be processed between 140 and 160° F and mixed The heating before dipping will vary with the coating It is important to keep all moisture out of and away from the chocolate, otherwise it will darken, thicken and become granular H Pyenson

36. Wrapper for frozen confections. S B SMITH U S Patent 2,450,364 3 claims
Sept 28, 1948 Official Gaz U S Pat Office,
614(4) 1019 1948

Frozen confections on a stick are enclosed in a paper wrapper which may be peeled as con-

sumed, providing insulation and acting as a drip collector
R Whitaker

37. The use of liquid sugar in ice cream. H G DUNLOP Can Dairy Ice Cream J, 27, 7
35-36 July, 1948

Liquid sugar is an accepted standard ingredient in ice cream The savings amount to 30 to 45 cents per cwt No trouble has been encountered with fermentation Crystallization was eliminated by insulating the tanks The concentration of the sugar solids should not be more than 67% Storage is not a problem when planning and scheduling is done properly The amount of liquid sugar is measured by a meter on an auxiliary calibrated tank The quality of liquid sugar is high H Pyenson

38. Liquid sugar for use in ice cream. ANONYMOUS Ice Cream Rev, 31, 3 64, 66-68 Oct,
1948

The use of liquid sugar as a standard ingredient of ice cream is increasing among the larger plants located near a source of supply The installation of equipment for the efficient handling of liquid sugar usually is justified if the annual consumption of sugar is not less than 250,000 lb The customary method of delivery is by stainless steel trucks with a capacity of 1,500 to 2,500 gallons or by tank cars holding from 6,000 to 10,000 gallons Liquid sugar may be obtained as a pure water solution of sucrose, sucrose and invert sugar, or sucrose and corn syrup If invert sugar is present, the amount should not exceed 15% because of the effect of the invert sugar on the freezing point of the mix The liquid sugar for use in ice cream mix sometimes is measured by volume using special measuring tanks or meters A more accurate method is to weigh the syrup with a tank scale as it flows to the pasteurizer

Liquid sugar in ice cream saves time and labor in handling, conserves valuable storage space since the storage tanks for liquid sugar can be located in areas where space is not at a premium, assists in streamlining plant operations, is more sanitary and offers less chance for waste or loss Limitations of the product are the higher transportation costs because of the water contained in the product and the requirement of 2 storage tanks of large capacity in most cases to insure providing an adequate supply of the product on hand at all times

W. J Caulfield

39. Flavor materials. CARL KOFVRFR, Pioneer Ice Cream Div, The Borden Co, Brooklyn, N Y

Ice Cream Trade J., 44, 10: 84, 164-173. Oct., 1948.

Coffee flavored ice cream is very popular in the New England States. It is consumed mostly by adults. Some coffee extracts have lost their caffeine content, and ice cream flavored with these is safe for children to eat. No added color should be used since it detracts from the coffee flavor.

The amount of chocolate flavor will vary in different localities. Three to 4% by weight of cocoa and up to 7% of liquor chocolate may be used. This amount of chocolate flavor will add 0.5 to 3.5% cocoa butter to the ice cream; too much cocoa fat gives the ice cream a gummy and sticky body. About 3 quarts of chocolate syrup are used to make 5 gallons of mix. Chocolate ice cream needs more sugar than vanilla; sometimes 18 to 20% sugar is used. Milk solids may be reduced to 8 or 9% and the stabilizer cut one-half. From 4 to 6% chocolate flavor should be used. Straight liquor chocolate is not recommended.

Strawberries for ice cream should be packed with 1 part of sugar to 3 parts of berries; usually Marshal berries from Oregon and Blakemore and Missionary from the South are used. Care must be taken to make sure that all of the sugar is dissolved before the berries are frozen. Strawberry ice cream and most fruit ice creams can stand 19 to 21% sugar. It may be desirable to make a special mix for strawberry ice cream containing 12% fat, 12% serum solids and 12% sugar with highest limit of stabilizer. Seven gallons of this mix, and 3 gallons of 2 to 1 pack strawberries will give a combination testing 8.4% fat, 8.4% solids-not-fat and 20.5% sugar. Red raspberry puree, 3 to 1 pack, of grade B or better berries is recommended. The amount needed to flavor ice cream is 3 quarts of puree to 34 to 37 quarts of mix. Seedless black raspberry puree with a sugar content up to 50% may be used.

Peaches require special care in their preparation for freezing. When lye is used in the blanch to loosen skins, the blanched peaches should be run through a cold citric acid bath. Ascorbic acid is added to retard oxidation and browning. After blanching, the peaches should be cooled in very cold water. The amount of peaches necessary to impart a characteristic flavor to the ice cream is large, usually 20 to 35% of a 3 to 1 pack. A peach puree, made from whole pitted peaches with the skins, may be used in combination with fresh peaches, using 0.67 peaches and 0.33 puree. Use of green peaches should be avoided. Artificial peach flavor some-

times is used in combination with peaches. July 4 to Labor Day is the best season for peach ice cream.

Pineapple contains an enzyme which may produce a bitter almond and a "fishy" flavor, unless destroyed by heat. A 2.5 to 1 pack used at the rate of 2 to 2.5 gallons of pineapple to 8 to 7.5 gallons of mix is recommended. Sterilized canned pineapple is preferred by some manufacturers.

Montmorency sour pitted cherries are not satisfactory for ice cream. Black Bing and Royal Ann cherries, especially processed for ice cream, may be used. This ice cream is sold under the name of Whitehouse or Cherry Vanilla. Five to 6 quarts of drained cherries are used for each 10 gallons of mix.

For banana ice cream, a cold pack puree using 15 lb. to 10 gallons of mix is recommended. Since bananas brown readily, ascorbic acid, with or without citric acid, is added, and this does not affect the flavor of the ice cream.

Various nuts and candies are added to the white mix as flavor. Variegated or ripple ice cream rapidly is becoming one of the best sellers. W. H. Martin

40. The effect of some emulsifying agents on the physical-chemical properties of ice cream. J. J. SHEURING, Univ. of Ga., and H. PYENSON, Univ. of Ill. *Southern Dairy Products J.*, 44, 5: 104-105, 107-109, 114. Nov., 1948.

The emulsifying agents studied, glycerol monostearate, sorbitan monostearate and mannitol ester of stearin, react as fat in the fat tests, but 0.1 to 0.3% was not detected by modified Babcock methods and in a minority of the cases was not detected by ether extraction methods. These products do not have any preservative effect and do not produce any marked differences in pH, titratable acidity or mix viscosity. They reduce whipping and freezing time 10 to 25% and increase shrinkage. Emulsifying agents apparently offer no protection against heat shock. The decreases in body and texture scores in storage were less when gelatin was used with emulsifying agents than when the latter were used alone. The use of a stabilizer with an emulsifying agent is recommended to get the maximum efficiency from both.

F. W. Bennett

41. The advantages of emulsifiers in the manufacture of ice cream. H. L. CASLER, Germantown Mfg. Co., Philadelphia 31, Pa. *Southern Dairy Products J.*, 44, 3: 28, 32. Sept., 1948.

Emulsifiers, like natural fats, belong to a

group of compounds known as esters, which are composed of fatty acids combined with alcohols. They differ from butterfat in that they contain more complex alcohols. If all the alcohol linkages are not taken up by fatty acids, emulsifiers have a greater affinity for both fat and water. Emulsifiers coat the homogenized fat globules and thus help to prevent clumping. Surface tension, the force which tends to pull the fat globules together to form larger masses, also is reduced. An emulsifier aids but cannot take the place of a stabilizer. Very few esters can be used as ice cream stabilizers. The proper amount of a suitable one must be determined by trial for each ice cream formula.

F. W. Bennett

42. The control of finished products in the ice cream plant. J. M. BROWN, H. P. Hood & Sons, Boston, Mass. *Southern Dairy Products J.*, 44, 3: 108, 110, 112-113. Sept., 1948.

In the control of finished products in the ice cream plant, the route salesman makes his request for products wanted in duplicate. The original is used by the chestman to supply the goods. The duplicate is used by the cashier in settlement and by the shipper in loading. The chestman releases stock only on orders written on designated forms. The products are checked on to the truck with the route salesman. The route salesman is required to settle daily for all products in his possession. Discounts are confirmed by an approved customer list. A summary of all products shipped, transferred or sold is made daily and serves as part of the balance control report.

F. W. Bennett

43. Full utilization of the ice cream plant laboratory. H. D. McAULEFFE. *Can. Dairy Ice Cream J.*, 27, 7: 70-76. July, 1948.

Ice cream making no longer is an art but a science. Technological advances in the ice cream industry have been able to standardize the product to a great extent. Most plants have laboratories, but very few dairies utilize them fully. The time spent in diagraming the flow of materials through a plant and deciding exactly what samples are needed is well worth while. The standard plate count simply is a device to determine whether or not the other methods of control are working. Many laboratories can expand their functions. Examinations can be made on fruits, nuts, stabilizers, sugars, fuels, paints and other materials purchased if practical for the larger operations. The laboratory can be used as a part of the public relations program.

H. Pyenson

44. Combination offer. ANONYMOUS. *Ice Cream Rev.*, 32, 3: 46, 146. Oct., 1948.

A special combination offer consisting of a half gallon container of ice cream, an ice cream dipper and 24 ice cream cones priced to sell for \$2.38 has been effective in building up carry-out sales. This combination of the merchandise was offered to the consumer for the first time during the month of July. It is reported that July sales on half gallons of ice cream were 73% greater than June sales on this item.

Display cards showing the items included in the combination offer and the price saving resulting from purchase of the combination were used to demonstrate and sell the idea to dealers and consumers. Dealer support of the combination offer was gained by capitalizing on the point that by encouraging customers to dip their cones at home the dealer would be able to reduce his sales on this short profit item.

W. J. Caulfield

45. Public relations for promoting dealer-consumer understanding of the ice cream industry. IRA MOSHER. *Ice Cream Trade J.*, 44, 9: 34, 69-72. Sept., 1948.

A survey conducted in New Jersey for a group of New York City and New Jersey ice cream manufacturers revealed that the majority of the retail dealers felt that they were being exploited by the manufacturers. Monthly news letters are being sent to some 8,500 dealers in an attempt to gain their confidence and expel this mistrust. These letters contain factual information on costs, profit possibilities, advantages of dealers, manufacturers cooperation, sanitation and prices.

W. H. Martin

46. Gallonage. V. M. RABUFFO. *Ice Cream Trade J.*, 44, 9: 32, 73-74. Sept., 1948.

Statistics released by the U. S. Bureau of Agricultural Economics show that ice cream production for the first 6 months of 1948 for the U. S. is running about 9% below that of 1947. In 2 of the states with the largest production, Pennsylvania and New York, production for the 6 months period was 4.4 and 6.6% below that of last year.

W. H. Martin

47. Sales promotions and stimulators in merchandizing ice cream. H. A. QUITTER. *Can. Dairy Ice Cream J.*, 27, 6: 48. June, 1948.

Helpful sales promotions and stimulators in merchandizing ice cream are (a) bonus plans, (b) animated window displays, (c) bright cano-

pics, fluorescent lights and pictures, (d) proper servings and (e) bulk dipped ice cream.

H. Pyenson

48. Costs, a comparative analysis for a 5 year period. J. SHIPLEY, Abbotts Dairies, Philadelphia, Pa. *Ice Cream Trade J.*, 44, 10: 88, 162-163. Oct., 1948.

Costs of manufacturing and sale of ice cream in the North Atlantic district increased from 96 cents per gallon in 1941 to \$1.16 in 1946. Most of the increase was due to higher products costs, which increased 62.2%, and greater manufacturing expense, which increased 4.9%. There were decreases of 24.3% in delivery, 15.6% in advertising and 29.9% in sales expense. The total net increase was 20.7% for the period.

W. H. Martin

MILK

P. H. TRACY, SECTION EDITOR

49. The effectiveness of milk ordinances. C. J. BABCOCK. *Can. Dairy Ice Cream J.*, 27, 7: 50-52. July, 1948.

Sanitation in milk production has not kept pace with the developments in plant sanitation. The sanitary control of the milk supplies of the U. S. ranges from practically no control to excellent control. Lack of enforcement and subterfuge in enforcement are responsible for a large percentage of the so-called grade A pasteurized milk that originates from raw milk containing a million or more bacteria per ml. The low salaries paid for milk control work contribute to the non-enforcement of milk ordinances. A majority of the milk ordinances on the statute books in the U.S.A. are not fully enforced. Therefore, it is impossible to judge a milk supply by the ordinance under which it is produced and handled.

H. Pyenson

50. Consumer evaluation of milk. W. A. WENTWORTH, The Borden Co., New York City. *Milk Dealer*, 37, 11: 46, 94-98. Aug., 1948.

Dairy prices are relatively low. Retail prices of fluid milk and cream throughout the U. S. have not increased over prewar as much as have most of the other principal foods. The increase in the per capita use of milk and cream over prewar is not as much as with many other foods. Dairy products are economical to buy. The author concludes that to avoid consumer criticism and resistance to prices for all dairy products which will equalize the competitive production situation on the farms of this country,

consumers must have a better realization of the money value of dairy products in comparison with other foods.

C. J. Babcock

51. Driver-owned milk routes. ANONYMOUS. *Milk Dealer*, 37, 9: 52, 118. June, 1948.

Shelly Dairy Co., Lima, Ohio, completed 2 years of operations under a system whereby drivers own their own trucks, maintain them and in most cases keep their trucks at home overnight. They pay Shelly Dairy Co. 16 cents a quart for milk at the platform, and sell it at a delivered retail price of 20 cents, thus operating on a margin of 4 cents.

C. J. Babcock

52. Tarboro's municipally-owned milk plant. J. M. GREGORY, Whitaker, N. C. *Milk Dealer*, 37, 12: 51-52, 88-90. Sept., 1948.

A description is given of the only city-owned dairy in the country. It is in Tarboro, N. C., a town of 10,000 population. The building is of brick construction 50 by 80 feet, 2 stories in front, 1 in the rear and cost \$20,000. The equipment is less than 2 years old and is valued at approximately \$16,000. About 500 gallons of sweet milk, 100 gallons of buttermilk and 100 gallons of chocolate milk are processed each day. About 200 gallons of sour cream are bought for butter and about 50 gallons of whipping cream are sold each week. The plant has been running successfully since 1918.

C. J. Babcock

53. Reducing operating costs with universal bottles. E. THOM, Assoc. Ed., *The Milk Dealer*. *Milk Dealer*, 37, 9: 48, 110-115. June, 1948.

Some individuality and advertising value is lost to a milk dealer when the universal glass bottle is adopted in a market. In the face of rising costs, however, interest in the universal bottle as a means of reducing those costs is increasing. The universal bottle program saves the distributors many thousands of dollars yearly, but those savings are not as important as the fact that the program promotes a healthy market condition.

Akron, Ohio, is the latest market to adopt the universal bottle, and information on unit costs or bottle trippage is not available. In the Columbus, Ohio, market, the 1947 cost was \$0.962 per 1000 units filled and the trippage on quarts was 36.6; on half-pints, 40.8; and the overall trippage for all bottles was 38.3. In Nashville during 1947 the market wide trippage was 35.57 and the unit cost was 1.2 mills. In Ft. Wayne, the dealers still were changing from one bottle to another, but the market had a

trippage of 34 on quarts, 24 on pints and 37 on half-pints. This gave a fillage cost of \$0.0069. None of the above markets have a universal case.

C. J. Babcock

54. Operation of the universal milk bottle plan in the Columbus market. W. K. HOLM, Columbus Milk Distributors Assoc., Columbus, Ohio. *Milk Dealer*, 37, 11: 41, 74. Aug., 1948.

Surveys have shown that the container cost ranges from 0.06 cent to over 1 cent per unit filled, which is from 0.3 to 5% of the total cost. The main factors affecting container costs are the original cost of container, the number of times a container is used, the deposit on a bottle if not returned, time consumed sorting foreign bottles, and the expenses involved in getting strayed bottles back.

The low bottle cost experienced with the universal bottle plan is due to: (a) Purchase of bottles in larger quantities and thus at a lower price when all dealers are using the same bottle. This saving is from 65 cents to over \$4.00 per gross, except for dealers who already are buying in large enough quantities to receive the lower price. At the present time one obtains the lowest price per gross if buying 3,000 gross a year. (b) The exchange of bottles is eliminated, saving time, storage space and transportation of bottles. (c) The elimination of the exchanging saves considerable wear and breakage of bottles. (d) More effective returns on the bottles from the consumer results. (e) Fewer bottles are tied up in inventory. (f) A uniform bottle deposit plan is used.

In addition to lower costs, other advantages are: (a) Grocers and milkmen are not bothered with more than one type of bottle. (b) Housewives have less difficulty returning bottles. (c) Such a plan tends to promote better relations between competing dealers.

The one single disadvantage to the universal bottle plan is that the individual advertising value of a dealer's own bottle is lost.

C. J. Babcock

55. Consumers' acceptance of the paper milk bottle. R. C. MILLER, Country Charm Dairy, Monticello, Ill. *Milk Dealer*, 37, 11: 102-106. Aug., 1948.

A questionnaire was placed in the hands of about 500 consumers. Among the advantages of paper bottles which were mentioned, 84 reported more sanitary, 80—easier to dispose of than wash and return bottle, 71—no bottle washing, 57—saves storage space, 53—more convenient, 46—no bottle return, 45—much safer to handle, 10—

lighter than glass, 5—no bottle accumulation, 4—no bottle deposit, 4—pour off more cream than glass, 3—easier to open than glass, 2—makes good frozen food storage containers, 1—protects milk from light and 1—will not freeze and break if housewife is not home. Among the disadvantages, 7 people mentioned paraffin scrapes off and falls in other food, 5—hard to pour, 3—hard to open, 3—dogs carry away, 2—paraffin taste, 2—can't clean outside well before storing, 2—can't pour out all the milk and 2—bottles not always full.

C. J. Babcock

56. Milk cans versus tank trucks. G. D. ARMERDING. *Can. Dairy Ice Cream J.*, 27, 7: 40-42. July, 1948.

The tank method is not practical for farms producing less than 300 gallons of milk per day. The advantages of the tank system are: (a) effective cooling with less risk of temperature rise; (b) eliminates all contact with oxidizing metals; (c) lower temperatures can be maintained while the product is in transit and all danger of freezing is avoided; (d) air-bourne contamination is eliminated; (e) no milk needs to be rejected due to high temperature spoilage; (f) spillage and sticking losses are avoided; (g) requires a minimum of hard labor on the farm and in the plant; (h) saves space on the farm and in the dairy; (i) makes possible every other day pick-up; (j) better control can be maintained by the dairy inspector; (k) the farmer can check his own weights on the farm; (l) elevated highways at milk plants no longer would be necessary and (m) the noise of can handling would be eliminated.

H. Pyenson

57. Top milk extractor. F. H. VALETON. (one half assigned to C. D. Colbert.) U. S. Patent 2,450,313. 2 claims. Sept. 28, 1948. *Official Gaz. U. S. Pat. Office*, 614(4): 1007. 1948.

The top milk is removed from a bottle of milk by introducing a liquid through a tube extending beneath the cream layer. The top of the bottle is sealed except for an outlet from which the top milk is collected.

R. Whitaker

58. Economics of milk cooling. L. P. BLAUSER, Ohio State Univ. *Milk Dealer*, 37, 11: 110-112. Aug., 1948.

The results of an Illinois experiment where samples of milk were held at different temperatures for a period of 12 hr. are reported. At 40° F. there was no increase in bacteria. At 50° F. the increase was slight. At 60° F. each bacterium produced 15 new ones. At 70° F. each

bacterium produced 700 new ones. At 80° F. each bacterium produced 3,000 new ones. The efficiency and cost of cooling milk with water, ice and mechanical refrigeration are compared. The author concludes that when the requirement for milk to be cooled to 50° F. more generally prevails, the use of electric milk coolers will become more universal, for it is the exceptional case where a well or spring has water of sufficiently low temperature to cool milk to 50° F.

C. J. Babcock

59. Homogenized milk problems. P. H. TRACY, Dept. of Food Technol., Univ. of Ill., Urbana. *Milk Dealer*, 37, 11: 49-54. Aug., 1948.

The author points out that, next to pasteurization, the process of homogenization probably has done more than any other thing to increase the popularity of milk as a beverage. The process of homogenization is described. Fat rising in homogenized milk, curd tension reduction, sediment control, homogenization of extra rich milk, control of the bacterial content, testing for fat and flavor control are discussed.

C. J. Babcock

60. Fat free milk as a special dairy product. C. P. SEGARD, Wis. Alumni Research Foundation, Madison. *Milk Dealer*, 37, 12: 55, 106-110. Sept., 1948.

Fat-free milk as a special dairy product and its use in the diet is discussed. Fluid milk and its by-products long have been recognized as the leading food product. There are many conditions recognized by physicians and surgeons where a high protein diet is required, and a high protein-low fat diet is easily acquired by the use of fat-free milk. It is recommended that vitamin A and D be added to the fat-free milk to offset the loss in the fat and to assure an intake of these vitamins that will offset any tendency toward deficiency. Since the medical profession has repeatedly shown the need for such a product, the Medical Milk Commission would seem to be the logical group in whose hands to place the quality responsibility. Those interested in the production of certified milk and those interested in the nutritional importance of fat-free certified milk may become responsible for such a product fortified with 4,000 U.S.P. units of vitamin A and 400 U.S.P. units of vitamin D.

C. J. Babcock

61. How to make a high quality chocolate-milk or dairy drink. B. P. FORBES, Benjamin P. Forbes Co., Cleveland, Ohio. *Milk Dealer*, 37, 10: 82-84. July, 1948.

The effect of a variation in milk solids on the

quality of chocolate milk and ways of increasing its flavor are discussed. It is pointed out that a good chocolate milk drink, one that will repeat and be a trade builder, should have a mild, pleasant milk chocolate flavor, little or no sedimentation, low to medium viscosity, light to medium color and medium sweetness.

The 2 most frequent defects in chocolate milk are settling and thickening or gelling. Settling may be caused by: (a) insufficiently stabilized cocoa for the amount of milk base to be processed; (b) too much milk or insufficient butterfat content for the amount or type of chocolate flavor used; (c) dilution of dairy drink by milk or water left in lines from pasteurizer to bottler; (d) restriction of valves on pressure side of pumps, causing a homogenizing effect of excessive whipping by centrifugal pumps; (e) too acid milk (keep acidity below 0.15%, but not by neutralizing); (f) using soda-neutralized milk; (g) using frozen milk (also causes wheying off); (h) incorrect pasteurization and cooling; (i) insufficient heat or too short holding time; (j) over agitation during and after cooling; (k) powder not entirely incorporated in the milk base; (l) not cooled to 40° or lower; (m) precooling below 140° in vat.

Thickening or gelling may be caused by: (a) excessive pasteurizing temperature; (b) too much stabilized chocolate flavor used, causing over stabilization; (c) use of lime or magnesium neutralizers; (d) improperly rinsed bottles; (e) too high acid milk.

C. J. Babcock

62. Recent technological advancements in the dairy industry. J. H. HETRICK, Dean Milk Co., Rockford, Ill. *Milk Dealer*, 37, 9: 156-162. June, 1948.

The dairy industry has been the beneficiary of many phases of chemical, bacteriological and engineering developments and "know-how". The application of scientific knowledge in many interrelated fields has made it possible for the industry continually to improve its products, to increase efficiency in production and distribution and to meet the increasing demand for these products. New products and new uses for old products have been developed. The keeping quality of the products has been improved through newer knowledge of processing, packaging and storage and through improved control practices. The nutritional properties have been enhanced. Methods for utilization of surpluses have been discovered to the economic advantage of the industry and the consumers of its products.

One of the most important essentials in the production of good milk is clean sterile milk handling utensils and equipment. Research has

developed cleaning aids specifically designed to handle milk wastes, and effective chemical sterilizers for the job. Research has produced analytical tools and a better understanding of the analytical methods used, such as the methylene blue test, the resazurin test, plate and direct microscopic bacteria counting, the sediment test, etc. These aid in assessing milk quality at the plant.

The biggest factor that led to the acceptance of pasteurization was definite proof that numerous epidemics of contagious diseases frequently were traced directly to contaminated milk. It also was well demonstrated in the laboratory that proper pasteurization would do the job of destroying the organisms responsible for the disease. The development of more efficient heaters, sensitive control devices and research on bacterial destruction brought about the approval and use of high temperature short time continuous pasteurization. Studies in ultrasonics, X-radiation and ultra violet radiation, etc., may culminate in improved sterilized dairy products. Research on the nutritional properties of milk has led to vitamin D milk which today is accepted as one of the most outstanding contributions of dairy research to public health. Research on homogenized milk has been responsible for the rapid acceptance of this product. Technological know-how has been used in the manufacturing, packaging and storage of dried milk, dried ice cream mix, dried cream, sterilized dairy drinks, frozen milk, frozen concentrated milk, butter oil and army spreads. There has been an increased realization of the necessity for and the value of scientific research, development and laboratory control.

C. J. Babcock

Also see abs. no. 8, 9, 10, 11, 14, 15, 18, 20, 25, 27, 34.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

63. Effects of steroids on lactation. J. C. BARSANTINI, AND G. M. C. MASSON, Univ. of Montreal. *Endocrinology*, 41, 4: 299. Oct., 1947.

Female albino rats weighing 250-300 g. and fed Purina Fox Chow were used. Within 24 hr. of parturition, the litters were either reduced or brought up to 6. Steroid treatment was started immediately, and some of the rats were ovariectomized. Efficiency of lactation was estimated by the gains in body weight of the young and by the number of deaths among the young. In normal rats estradiol at daily doses of 10 γ and 1 mg., and testosterone, androstene-

diol, dehydro-iso-androsterone at a daily dose of 10 mg. caused a marked inhibition of lactation. At the daily dose of 10 mg., androstenedione, methyl androstenediol, methyl androstenediol, ethyl testosterone, ethynyl testosterone, ethynyl androstenediol and methyl testosterone produced a definite but much less marked inhibitory effect. Desoxy-corticosterone, pregnenolone, acetoxypregnenolone, and progesterone were inactive. All of the steroids were inactive in spayed lactating rats.

Ralph P. Reece

Also see abs. no. 66.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

64. Clinical and postmortem observations on metrorrhagia in the virgin heifer. A. F. WEBER, B. B. MORGAN, AND S. H. McNUTT, Univ. of Wis., Madison. *North Am. Veterinarian*, 29, 11: 705-710. Nov., 1948.

Sixty-eight complete estrus cycles were studied in 22 clinically normal virgin heifers. Several estrus cycles were observed in each heifer, and they then were slaughtered during proestrus, estrus and the first 4 days post estrus and used for postmortem studies. Uterine motility was studied by rectal palpation. Evidence of uterine bleeding was macroscopically observed in 55 of the 68 cycles followed. Vaginal swab studies showed the presence of microscopic bleeding in the remainder of normal estrus periods. The average time of appearance of uterine bleeding in the 55 macroscopic cases was 36 hr. after the onset of estrus. Endometrial edema, as determined by postmortem studies, began during early proestrus and reached a maximum at the time of ovulation. Rectal palpation revealed regular uterine contractions during the interestrus and estrus periods, with maximal contraction at the time of estrus. Following ovulation the uterus was in a quiescent condition for several days with only irregular partial muscular contractions.

R. P. Niedermeier

65. Effect of adrenalectomy on the mammary gland of the castrated and estrogen treated castrated male rat. J. J. TRENTIN, AND C. W. TURNER, Univ. of Mo. *Endocrinology*, 41, 2: 127. Aug., 1947.

In Wistar male rats weighing from 200 to 300 g., castration resulted in a reduction but not complete elimination of the alveolar development normally found in the mammary glands of adult males. The removal of the adrenals of castrated male rats, with maintenance on 1% saline for 10 days, resulted in atrophic ducts and in com-

plete regression of the alveolar development. In adrenalectomized, castrated rats maintained on salt the daily administration of 5 γ of α -estradiol benzoate for 10 days, although causing noticeable duct stimulation, was relatively ineffective in stimulating alveolar growth as compared with castrated rats.

Ralph P. Reece

66. The role of the adrenal cortex in mammary development and its relation to the mammogenic action of the anterior pituitary. A. T. COWIE AND S. J. FOLLEY, Nat. Inst. for Research in Dairying. *Endocrinology*, 40, 4: 274. April, 1947.

Immature hooded Norway rats of both sexes, gonadectomized either at weaning or a few days thereafter, were used. In 1 experiment, the adrenals were removed at the time of gonadectomy; in 2 experiments adrenalectomy was postponed until some weeks after weaning. Adrenalectomized rats received saline for drinking water. Some rats received daily subcutaneous injections of a saline extract of ox anterior pituitary, 1 ml. of which was equivalent to 250 mg. fresh tissue. A semi-quantitative method of scoring the kind and degree of glandular development was devised and used. Regressive changes in the mammary glands frequently were observed following adrenalectomy; increased arborescence of the duct system never was observed. The pituitary extract showed mammogenic activity both in the presence and absence of the adrenals, duct growth being most prominent in the youngest rats, while in older rats stimulation of alveolar development also occurred. Adrenalectomy sometimes altered the mammogenic effects of the anterior-pituitary extract; however, the results indicated that the mammogenic action of the extract was not due primarily to its stimulation of the adrenal cortex. Alveoli eventually developed in the mammary glands of untreated rats gonadectomized when sexually immature.

Ralph P. Reece

67. Hyaluronidase levels of rat testes as related to age, hypophysectomy and cryptorchidism. S. L. LEONARD, P. L. PERLMAR, AND R. KURZROK, Cornell Univ. *Endocrinology*, 42, 3: 176. March, 1948.

Hyaluronidase determinations were made on testes of rats of varying ages by the turbidimetric method and by the rat-ova test. Testes homogenates were prepared in acetate buffer at pH 6 for the turbidimetric tests and in Ringer's solution for the rat-ova test. The amount of enzyme per g. of tissue gradually increased between 21 d. of age and sexual maturity. The hyaluronidase level was low by the tenth day following experi-

mental cryptorchidism and by the 24th day after hypophysectomy. Enzyme levels and the degree of development of the germinal epithelium were correlated directly. All of the hyaluronidase was not accounted for by mature sperm, since it was detected in testes devoid of sperm.

Ralph P. Reece

68. Effects of vitamin A deficiency on thyroid function studied with radioactive iodine. M. B. LIPSETT, AND R. J. WINZLER, Univ. Southern Calif., School of Medicine. *Endocrinology*, 41, 6: 494. Dec., 1947.

Using radioactive iodine as a tracer, the functioning of the thyroid gland of the rat was investigated in severe avitaminosis A. The thyroid glands of vitamin A deficient rats were relatively heavier than in control animals and the histological picture showed degenerative changes and distended follicles present within the same gland. The total I^{131} uptake of thyroid glands from vitamin A deficient rats was similar to that of glands from control rats. The percentage of inorganic I^{131} in the thyroid gland of vitamin A-deficient rats reached higher values and decreased more slowly than in the controls. There was a significantly lower rate of thyroxine formation in the vitamin A-deficient rats and a decreased final level at 96 hr.

Ralph P. Reece

Also see abs. no. 30, 63.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

69. Laboratory procedure for evaluating practical performance of quaternary ammonium and other germicides proposed for sanitizing food utensils. G. R. WEBER AND L. A. BLACK, U. S. Public Health Service, Cincinnati, Ohio. *Am. J. Pub. Health*, 38, 10: 1405-1417. Oct., 1948.

Five ml. of a suspension of a test organism containing 200 million organisms per ml. was mixed with 5 ml. of the germicide whose concentration was twice that desired for testing. The time required to obtain a 100% kill of the organisms by the germicide used indicated the exposure time required for sanitizing food utensils. The test organisms used were *Escherichia coli*, *Staphylococcus aureus* and *Micrococcus caseolyticus*. *E. coli* was preferred as the test organism, since the others gave a reduced killing time. Using distilled water instead of tap water to prepare the germicidal solutions also shortened the killing time. An alkaline hypochlorite of 50 p.p.m. available chlorine gave 100% kills in 30 seconds, while a chloramine T solution of 200 p.p.m. required 120 seconds. Some quaternaries gave a

100% kill of *E. coli* in 60-120 seconds when recommended concentration, usually 200 p.p.m., was used. Other quarternaries did not give complete kills even after 5 min. exposure.

D. D. Deane

70. The effect of quaternary ammonium sanitizers on the quality of milk. A. V. MOORE, Texas A. & M. College. Southern Dairy Products J., 44, 5: 62-64. Nov., 1948.

Fresh raw milk in 100 ml. quantities was treated with from 0 to 175 p.p.m. of 3 well known quaternary ammonium sanitizers. A duplicate set of samples was prepared and 2 ml. of active starter added to each sample. All samples were incubated at 70° F. and examined after 20 and 40 hr. After 20 hr. the titratable acidity of the samples without starter or sanitizer was 0.68 to 0.71%. The acidity of the samples containing 25 p.p.m. of quaternary sanitizer ranged from 0.51 to 0.67%, while the acidity of those containing from 50 to 175 p.p.m. varied from 0.30 to 0.20%. The samples containing 2 ml. each of starter without sanitizer had an acidity of 0.85 to 0.86%. Those containing starter and 25 p.p.m. of a quaternary sanitizer varied from 0.50 to 0.70% in acidity while the acidities of those containing from 50 to 175 p.p.m. ranged from 0.41 to 0.24%. All except 1 of the 36 samples containing 50 p.p.m. or more of a quaternary sanitizer had musty, putrid or stale odors. After 40 hr., the samples containing 25 p.p.m. of a quaternary sanitizer, with or without starter, had acidities ranging from 0.42 to 0.86%. The acidities of all samples containing from 50 to 175 p.p.m. of a quaternary sanitizer ranged from 0.69 to 0.23%.

The samples containing 25 to 175 p.p.m. of available chlorine all were normal in odor and acidity except 2 without starter which had a chlorine odor. The addition of sanitizers to milk is very detrimental to the quality of the milk, as well as being an illegal practice.

F. W. Bennett

71. Present status of chemical sterilizers. D. V. JOSEPHSON, Dept. of Dairy Husb., Penn. State College. Milk Dealer, 37, 12: 102-104. Sept., 1948.

Chlorine sterilizers are compared with the quaternary ammonium compounds. The latter compounds have certain advantages in that they are non-toxic, odorless, non-corrosive, stable to heat and air, not greatly affected by alkaline conditions, not as reactive with organic matter and have wetting and penetrating powers. They have certain disadvantages in that the various types are

not equally effective, and reliable and simple means of analysis have not yet been developed. Furthermore, the quaternary agents are liquids and are more costly to use. They are not compatible with soaps, certain phosphates, silicates and anionic wetting agents. C. J. Babcock

72. Sanitizing milking machines. A. C. DAHLBERG. Can. Dairy Ice Cream J., 27, 7: 60-62. July, 1948.

Boiling water was an effective germicide but was hard on the rubber. Later a concentrated brine solution in a crock was used on the rubber parts as a disinfectant. Then chlorine was added to the salt brine. The solution racks have now replaced the crock and the chlorine has been replaced by 0.4% lye solution. More recently new water softeners and the complex phosphates aided in cleaning and preventing mineral deposits in the rubber parts. The nontoxic cationic germicides are more or less stable in nature and offer a new attack in sterilization, but some of them are not compatible with water softening compounds and surface active detergents. H. Pyenson

73. New developments in sanitizing teat cups. W. S. MUELLER AND D. B. SEELEY, Univ. of Mass., Amherst. Hoard's Dairyman, 93, 21: 807. Nov. 10, 1948.

One extra milker head assembly for every 2 complete milking machines used is recommended as a means of increasing contact time between teat cup and germicide. Germicidal solutions were made up to contain 400 p.p.m. Approximately 20 teat cups were examined for bacteria after being treated as follows: (a) before use after being stored in lye solution, (b) after milking 1 cow but not being rinsed in water or sanitized, (c) after rinsing in cold water and sanitizing for 1 sec. in the quaternary solution, (d) after rinsing in cold water and sanitizing for 2 min. in the quaternary solution. The extra milker head remained in the germicidal solution when not in use and was alternated from one machine to the other between cows. The teat cups were sanitized between cows milked for 2 minutes or more without increasing the milking time of the herd. Where the extra head was used the total bacterial count of the teat cup was reduced considerably and 99.7% of *Str. agalactiae* organisms were killed under laboratory conditions.

J. B. Frye, Jr.

74. Sanitation for the dairy farm. M. P. BAKER, Iowa State College, Ames. Milk Dealer, 37, 9: 98-104. June, 1948.

Quality milk is defined as meaning clean milk

of good flavor, of low bacterial content and containing no harmful microorganisms such as pathogenic bacteria. Clean cows, sterile utensils and prompt cooling are major factors in the production of milk with low bacterial content. Healthy cows, healthy, clean milkers and milk handlers, safe farm water supplies and protection from flies are factors that guard against undesirable types of contamination. These are not necessarily expensive. Many of the items important in the production of high quality milk also are important from the standpoint of efficiency of dairy farm operation.

C. J. Babcock

75. You can clean up with less. H. F. KERN, JR., Freeman's Dairy, Allentown, Penn. Milk Dealer, 37, 11: 43, 67. Aug., 1948.

The biggest waste of washing powder was due to the large bucket of powder that normally was kept in each department. It was too easily accessible, and there was a tendency to use a scoopful when a teaspoonful would have been sufficient. An educational program, including bagging the powder into convenient lb. packages

and dispensing it in correct amounts to each workman for his respective job, resulted in a reduction in the quantity of powder used.

C. J. Babcock

76. Prevention of insects in dairy plants. Ed. M. SEARLES, Sealtest, Inc. Milk Dealer, 37, 10: 48, 86-90. July, 1948.

The discussion may be summarized as follows: (a) keep insects out of plant, (b) clean out breeding and hiding places, (c) use 5% DDT in odorless kerosene by trained personnel, (d) use the proper sprayer, (e) spray only the insect resting, roosting and hiding places and (f) never let the spray fall into or onto food or into containers used for holding food.

C. J. Babcock

77. Forty years of progressive dairy sanitation. R. S. BREED. Can. Dairy Ice Cream J., 27, 7: 43-49. July, 1948.

The article discusses the progress made in dairy sanitation and gives an outline of determinative bacteriology.

H. Pyenson

Also see abs. no. 17, 22.

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEW

78. **Milk and dairy products.** L. M. LAMPERT. 291 pp. \$7.00. Chemical Publishing Co., Inc. 1947.

This book is intended as a summary of the more important points in the chemistry, bacteriology, and the technology of milk and milk products. The illustrations are numerous and add considerably to the value of the presentation. References to the literature permit one to obtain additional information on many of the points covered. The paper and printing are quite satisfactory. The one criticism the reviewer would make is that the brevity of treatment has been carried to the point that the reader might be left without a satisfactory appreciation of the importance of some of the points which are not covered. An example of this is the failure to make any statement about any of the less common varieties of bacteria which may be responsible for some of the more serious defects in the various dairy products. F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

79. **The early features of bovine mastitis.** J. F. MALCOLM and M. M. CAMPBELL. West of Scotland Agr. College, 6 Blythswood Square, Glasgow, C. 2. *Proc. Soc. Applied Bact.*, 1946, 1: 29-34. 1946.

Observations were made on heifers as they entered the milking herd, examinations being made fortnightly or weekly throughout the first and succeeding lactations. In subclinical cases of mastitis, a rise in the cell content was the first indication of the presence of the disease; milk from mastitis-free animals usually had a cell count of less than 100,000 and frequently less than 50,000. Causative bacteria, e.g. streptococci and staphylococci, frequently were not found in milk having high cell counts, although cows giving such milk showed clinical evidence of mastitis. It was postulated that the pathogenic bacteria often considered as the cause of mastitis may be only secondary invaders which are enabled to obtain a foothold owing to abnormal conditions initially caused by some unknown agent, specific or non-specific in nature. Basis for this also was claimed by the fact that non-specific mastitis in early lactations would in sub-

sequent lactations develop into definite infections. M. L. Speck

80. **Sulpha preparation for animal mastitis.** D. G. PERKINS. (Assigned to E. R. Squibb and Sons.) U. S. Patent 2,453,259. 3 claims. Nov. 9, 1948. *Official Gaz. U. S. Pat. Office*, 616, 2: 439. 1948.

A colloidal suspension of a chemotherapeutic agent of the sulphanilamide type in mineral oil is injected into the animal for treating mastitis. R. Whitaker

81. **Some observations on milk fever.** A. ROBERTSON, J. W. BURGESS, A. MARR, and BETSY J. C. MILNE. *Vet. Record*, 60, 42: 505-508. Oct. 16, 1948.

This study includes data on 25 cases of milk fever. Seventeen of the cases responded normally to calcium therapy, and blood levels for calcium, magnesium and inorganic phosphorus are tabulated for before treatment, soon after treatment and after recovery periods. The average before treatment calcium level was 5.4 mg % as compared to 11.85 mg % in the after recovery period. Magnesium increased slightly from 2.48 to 2.81 mg %, whereas inorganic phosphorus increased from 2.1 to 3.6 mg % in the same periods. In most cases that did not respond to calcium therapy, inflation was used successfully, and the inorganic phosphorus showed a marked rapid rise following inflation. The authors suggested that in some cases of milk fever the treatment effect upon the inorganic phosphorus level may explain the observations of practitioners that at times inflation is a better treatment for milk fever than calcium therapy. R. P. Niedermeier

BUTTER

O. F. HUNZIKER, SECTION EDITOR

82. **Water insoluble fatty acids in cream and butter.** J. HILLIG and S. W. AHLMANN, Food and Drug Admin., Federal Security Agency, Washington, D. C. *J. Assoc. Offic. Agr. Chemists*, 31, 4: 739-749. Nov., 1948.

An investigation was made of certain chemical changes taking place in cream constituents during initial lactic acid souring and in cream in which additional deteriorating changes had occurred. A relationship was found between the occurrence

of unidentified acids (acids other than lactic and volatile acids) in butter and the condition of the cream from which it was churned. The unidentified acids consisted chiefly of water-insoluble acids. When butter was made from decomposed cream, the quantity of water-insoluble acids increased materially. There was no significant change in the content of water-insoluble acids in butter during storage for 5 months at 0° F. The presence of mold tended to increase the water-insoluble acids in butter. Experiments designed to study the partition of water-insoluble acids between butter and buttermilk showed that most of the acids were retained in the butter, very little being found in the buttermilk. Individual cans of cream classified as being decomposed usually contained much larger quantities of water-insoluble acids than cans of cream classified as satisfactory for buttermaking.

F. J. Babel

83. Volatile acids in cream and butter. Part 1. The development of butyric acid during the progressive decomposition of cream. F. HILLIG, Food and Drug Admin., Federal Security Agency, Washington, D. C. **Part 2. Butyric acid in commercial creams and butters.** F. HILLIG and DOROTHY MONTGOMERY, Food and Drug Admin., Federal Security Agency, Washington, D. C. J. Assoc. Offic. Agr. Chemists, 31, 4: 750-760. Nov., 1948.

Experiments on the decomposition of cream showed that butyric acid frequently was produced in cream when it had reached a point of being classified as unfit for human consumption. The butyric acid was thought to result from the breakdown of lactose and not from the hydrolysis of the fat. When butyric acid was present in cream, a portion usually was carried over into the resulting butter. When the amount of butyric acid present in butter was sufficient to be detected by the method employed (J. Assoc. Offic. Agr. Chemists, 28: 644. 1945), it was considered that the product had been made from some decomposed cream. An undeterminable amount of butyric acid in butter was considered as an indication that the product had been churned from sound cream. Butyric acid was not found in commercial sweet cream butter.

F. J. Babel

CHEESE

A. C. DAHLBERG, SECTION EDITOR

84. Mikroflora und Struktur des Reifenden Camembertkäses. (The microflora and structure of ripening camembert cheese.) English summary. G. ENGEL. Die Milchwissenschaft, 3, 7: 46-51. 1948.

Camembert cheese was examined during its first 3 weeks of ripening for (a) microbial population, (b) types of flora, (c) pH changes on the surface and within the cheese. Direct microscopic counts were made according to Breed, using Newman stain. Eight smears were prepared from a 10 g. sample and 10 fields counted on each smear. The pH was determined on cheese solutions saturated with quinhydrone and read against a calomel half cell electrode. The distribution of microorganisms in the cheese structure was observed, using a modified procedure of Bondioli which was conducted as follows: small pieces of cheese 0.5 cm. wide were held in a desiccator (containing sulfuric acid) at 4° C. from 1 to 4 days. The firmed cheese was cut 5 μ thick with a microtome, placed on a slide and treated with 1 drop of a mixture of alcohol-xylol-aniline oil. The excess liquid was removed with filter paper, and the dry preparation stained with Newman or Gram stain.

Slight peaks in population were observed at 1-2, 9-11 and 13-15 day old cheese. The first peak was due to acid-forming diplococci and streptococci, whereas the second peak was due to oidium, yeasts and *Penicillium album*, and the third peak was due to an increase in lactobacilli (*Lactobacillus casei*). The pH of the new cheese was the same throughout; the pH increased more rapidly in the outer layer than within, showing a difference of 1.74 units on the 21st day of ripening, namely pH 7.73 as against pH 5.99.

I. Peters

85. Process for making cheese. Z. D. ROUNDY and H. L. KEEL. (Assigned to Armour and Co.) U. S. Patent 2,450,814. 11 claims. Oct. 5, 1948. Official Gaz. U. S. Pat. Office, 615, 1: 218. 1948.

Cheese, ripened in less than 1 week, is converted into process cheese products by thoroughly incorporating inactive proteolytic pancreatic zymogen material and an activating agent, such as enterokinase, in the milk at setting time. After cutting and firming the curd in the conventional manner, the curd and whey are heated for 10 to 15 min. to 115° (range 110 to 120° F.) to activate the proteolytical enzyme. After the curd is drained and matted, it is cooled and held about 5 days at 35 to 40° F. It then is ready for processing into cheese products. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

86. Milk fat. How the product is manufactured and packaged. Its advantages and potential

uses. T. I. HEDRICK, North Star Dairy, St. Paul, Minn. Milk Plant Monthly, 37, 10: 80-83. Oct., 1948.

The use of milk fat in the dairy, confection and baking industries has increased tremendously in recent years. This product offers some advantages when compared to butter or cream in that it has a superior keeping quality and is less bulky, thus reducing storage and handling costs.

Different methods of manufacturing milk fat have been perfected, but the method most widely used in the U. S. involves churning of the cream followed by a removal of varying amounts of water and non-fat solids from the melted butter by a filtering, boiling or centrifuging process.

Packaging and storing present the greatest problem, since the fat is susceptible to oxidative and hydrolytic deterioration. The procedures advocated for preventing this decomposition of the fat are: (a) selection of a high quality raw ingredient, (b) avoiding contact of the product or raw materials with metallic catalysts, (c) pasteurization at 170° F. for 20 minutes, which destroys the milk enzyme lipase, (d) maintenance of a moisture content in the finished product under 0.3%, (e) deaeration of the finished product to an oxygen content under 1%, (f) storage in nitrogen packed, hermetically sealed cans when the product is to be held above refrigeration temperatures, and (g) the use of antioxidants such as wheat germ oil and N.D.G.A.

J. A. Meiser, Jr.

87. Plastic cream. Its production and uses. R. J. SPIERS, Abbots Dairies, Inc., Philadelphia, Penn. Milk Plant Monthly, 37, 10: 122-123. Oct., 1948.

To produce a high quality plastic cream, only raw materials of excellent quality can be used, and freedom from copper and iron contamination must be maintained throughout the manufacturing process. Pasteurization after the first separation should take place at 170° F. for not less than 15 min. This is followed by a second separation at 145° F. Packaging should be done only when the product is sufficiently fluid to completely fill the containers. Rapid freezing is essential, thus air blast equipment which circulates large volumes of air at a temperature of 10 to 20° F. below zero is ideal. If these procedures are followed, the product offers no laboratory control problems.

J. A. Meiser, Jr.

88. Manufacture, use and storage of dehydrated sweetened condensed skim milk. A. T. MUSSETT

and W. H. MARTIN, Kansas Agr. Expt. Sta., Manhattan. Ice Cream Rev., 32, 5: 44, 46, 48, 50. Dec., 1948.

A skim milk sugar solution containing around 14 to 22% solids is dried to a powder. The product is said to be more easily dispersed in ice cream mix than powdered milk, is easier to handle and requires less storage space than liquid sweetened condensed milk.

Experimental data pertaining to methods of manufacture indicate that the best powder from the standpoint of color, flavor and texture resulted when the temperature in the drying chamber was maintained at 325° F., with an atomizing pressure of 750 lb. per in.² and when the concentration of the liquid before drying was 14 to 22% solids. When a higher drying temperature (375° F.) was used, a lower moisture content resulted, but the powder prepared in this manner was less desirable from the standpoint of its color and flavor characteristics.

Ice cream prepared with sucrose-sweetened skim milk powder was found to be equal to the control samples with respect to flavor, body and texture and whipping ability. On the other hand, the use of maltose-sweetened powder increased the acidity of the mix, impaired the whipping ability of the mix and resulted in an ice cream characterized by a pronounced malt flavor. Both the maltose and sucrose powders were more easily and completely dispersed in the mix than either non fat dry milk solids or a mixture of pulverized non fat dry milk solids and sucrose.

Storage trials indicate that either glass or tin containers maintained the powders in better condition than when paper containers were used. Samples of the powder were stored for a period of 2 months at either 40° F. or at room temperature in tightly closed glass or tin containers without appreciable change in moisture content, acidity, solubility or flavor characteristics.

W. J. Caulfield

Also see abs. 101, 102, 114.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

89. Pasteurization and its relation to health. JOHN ANDREWS and A. W. FUCHS, U. S. Public Health Service, Washington, D. C. J. Am. Med. Assoc., 138, 2: 128-131. Sept. 11, 1948.

This is an excellent review paper, published at the request of the Council on Foods and Nutrition, Am. Med. Assoc., because of recent published attacks on pasteurization. There is presented a table of milk-borne disease outbreaks

reported during the years 1923-1945, comprised of 955 outbreaks, 40,177 cases, with 804 deaths. Obviously, reporting was not complete, and the data do not include sporadic cases of such diseases as typhoid fever, scarlet fever, septic sore throat, tuberculosis of bovine origin, infantile diarrhea, nor any significant amount of milk-borne brucellosis. In 1945 alone, 5,049 cases of brucellosis and 101 deaths were reported, and about half are believed to have been of raw milk origin. The risk of contracting disease from raw milk is approximately 50 times as great as from pasteurized milk.

Pasteurization is said to be the most important protective measure which can be applied to milk. The process is described briefly. It is stated that more than 75% of the market milk supply of American communities of over 1,000 population is pasteurized. Objections that have been raised to pasteurization are declared to be unsound. Much information is presented to refute claims of loss of nutritive value during pasteurization. The authors conclude that it is obvious that physicians and health authorities are justified in recommending that all milk be pasteurized.

D. P. Glick

90. A new method for fixing, defatting and staining milk and cream films. G. W. ANDERSON, R. MOEHRING, and N. O. GUNDERSON, Dept. of Public Health, Rockford, Ill. J. Milk and Food Technol., 11, 6: 352-357, 364. Nov.-Dec., 1948.

The authors suggest an improved method over the standard procedure for staining organisms in milk and cream. The slides are defatted and fixed in a chloroform-alcohol mixture for 1 to 2 minutes, dried for 2 minutes and stained with a polychrome methylene blue stain solution from 45° to 60 seconds. Fixing and defatting the milk film are combined in 1 step requiring from 3 to 6 minutes. Granulated particles on the slide are reduced to a minimum, this giving a uniform stained preparation. Decolorization after staining is not necessary since all the bacteria retain the dye. Detailed instructions and preparation of the reagents are given in the paper by the investigators.

H. H. Weiser

91. Some general aspects of the inhibitory streptococci. A. HIRSCH, Natl. Inst. Research Dairying, Univ. Reading. Proc. Soc. Applied Bact., 1946, 1: 26-28. 1946.

The antibiotic activity possessed by strains of *S. lactis* and *S. cremoris* presents certain features which are important both industrially and academically. The presence of such streptococci in starter cultures or their previous activity in milk intended for fermented products can be detected

by simple tests. The mode of action of the diplococci produced by such streptococci apparently is different from that of the sulfonamides and penicillin. The former acts very rapidly without lysis of the cell, indicating a lethal action at the cell surface; the latter act more slowly, indicating interference with anabolic activities within the bacterial cell.

S. lactis diplococci are active against *S. cremoris*, most other streptococci, other Gram-positive cocci and rods, mycobacteria and certain fungi. *S. cremoris* diplococci have little or no activity for organisms not belonging to serological group N.

M. L. Speck

92. Viability of *Brucella abortus* bovis in milk samples from infected cows. G. C. VAN DRIMMELEN, Inst. of Onderstepoort, Pretoria, S. Africa. J. So. African Vet. Med. Assoc., 19, 2: 58-60. June, 1948.

An intravenous injection of a field strain of *Brucella abortus* was made into a cross-bred Africander X Sussex cow. The cow aborted 1 week after the injection. Milk from this cow was used in the tests. Sediments from 100 cc. samples of milk stored in sterile test bottles on laboratory tables were injected in rats at daily intervals after samples were taken. Rats injected with the culture from milk stored only 1 or 2 d. contracted the disease, whereas the rats receiving the cultures from milk stored for 3 and 4 d. failed to show signs of *Brucella abortus*. The authors point out the futility of taking samples on the open market for *Brucella abortus* examination. It is suggested that milk samples should be taken under hygienic conditions. The samples should be packed in ice if required to ship to a distant laboratory.

K. M. Dunn

93. Fragen der Milchhygiene. (Questions concerned with milk hygiene.) English summary. B. KEMKES. Die Milchwissenschaft, 3, 4: 108-111. 1948.

The author lists sources of pathogens and shows the manner of their entrance into milk. After 25 years of eradication, 25 to 37% of Germany's milk cows still are infected with tuberculosis and 20% with brucellosis. He points out further the entrance of large numbers of non-pathogenic undesirable microorganisms into milk. Pasteurization is not a remedy for unsanitary produced milk. Rigid sanitary control and higher milk prices may result in a better quality milk.

The supervision of the milk supply is not a problem of the veterinarians only, but should be the duty of the physicians as well. But because the physicians are overloaded already with various duties, the responsibility of milk super-

vision should be turned over to hygienic bacteriological institutions, such institutions being well qualified for the task. I. Peters

94. The toxicity of certain organic acids to yeast and mold in the presence of fruit juice-syrup mixtures. R. E. MORSE, C. R. FELLERS and A. S. LEVINE, Dept. of Food Technol., Univ. of Mass., Amherst. J. Milk and Food Technol., 11, 6: 346-351. Nov.-Dec., 1948.

The preserving effects of acetic, citric and lactic acids and sodium benzoate were studied when each was combined with fruit juice-syrup mixtures. Acetic acid was more effective against mold than the yeast as compared to the other compounds, while yeast showed the reverse effect. Lactic acid required a concentration of 6.25% to stop yeast growth as compared to 4.58% to prevent mold development. Acetic acid was more toxic to the test organisms than either citric or lactic acids H. H. Weiser

95. Method of fermenting whey. E. R. ENGEL. U. S. Patent 2,449,064. 10 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 415. 1948.

A fermented alcoholic whey product is produced by culturing with yeast and sugar at 58 to 72° F. for 3 d., followed by a series of holding periods at lower temperatures, decantation of the liquid and final aging to produce a liquor of light green color, pleasant taste and bouquet and a milk smoothness. R. Whitaker

96. Chemical engineering unit processes review. Fermentation. H. E. SILCOX and S. B. LEE, Merck & Co., Rahway, N. J. Ind. Eng. Chem., 40, 9: 1602-1608. 1948.

This paper is one of 18 review articles on unit processes. Sections of the fermentation review of interest to the dairy industry include those on riboflavin, penicillin and lactic acid.

B. H. Webb

Also see abs. no. 83, 84.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

97. The cholesterol content of cows' milk. B. NATAF, O. MICKELSEN, A. KEYS, and W. E. PETERSEN. Univ. of Minn., Minneapolis and St. Paul. J. Nutrition, 36, 4: 495-506. Oct., 1948.

The cholesterol content of "winter" and "summer" milks from Holstein, Jersey and Guernsey cows was determined. No ester cholesterol was found. The average cholesterol content of milk

from all cows was 11.4 mg. per 100 ml. during winter and 11.3 mg. during summer. Holstein milk contained more cholesterol in summer than winter, but the reverse was true for Jerseys. These seasonal differences were barely significant. The data from Guernsey cows were less complete and a similar comparison was not made. The milk from Holsteins contained less cholesterol than the other breeds during the winter, but differences between breeds were not significant for summer milk because of comparatively large variations between cows of the same breed during this season.

Significant correlations were found between fat and cholesterol contents during both winter and summer. R. K. Waugh

98. Über den Nachweis der Kurzerhitzung der Milch mit Hilfe der Phosphatasereaktion. (On the phosphatase test for checking of short time heating.) English summary. G. SCHWARZ and O. FISCHER. Die Milchwissenschaft, 3, 2: 41-45. 1948.

Comparisons between the Scharer and the Kay and Graham phosphatase tests showed both tests to be equally sensitive. However, the difficulty in obtaining the rather expensive phosphotungstic acid and molybdate required in the latter test induced the authors to use the Scharer method. The Scharer test was modified in that the lead acetate was replaced by ZnSO_4 ; borax by Na_2CO_3 and NaHCO_3 , and by the addition of N/4 NaOH to adjust the reaction to pH 9.3. The modified Scharer test required the following reagents: (a) 1 g. disodium phenylphosphate (phenol free) together with 1 g. Na_2CO_3 and 9 g. NaHCO_3 , dissolved in water to make 1 l. Prepare fresh daily. (b) 10% ZnSO_4 ; (c) N/4 NaOH; (d) 10 mg. 2,6-dibromoquinonchlorimid in 10 ml. of 96% alcohol. Prepare fresh daily and keep away from light.

Procedure: To 0.5 ml. of milk add 10 ml. of buffer (a). Mix and hold for 2 hr. at 37° C. Add 1 ml. of 10% ZnSO_4 . Mix well and add 1.5 ml. N/4 NaOH and filter through hardened filter paper (Schleicher and Schull 605, extra hard). Add to the clear filtrate 0.5 ml. N/4 NaOH and 10 drops dibromoquinonchlorimid reagent. Hold for 15 minutes and determine extinction coefficient, using filter 561. By omitting the use of ZnSO_4 and by increasing the holding time at 37° C. from 2 to from 4 to 6 hr., positive tests were obtained (a) if milk was heated for 30 minutes at 61° C. instead of at 63° C., (b) if milk was heated at 62° for 20 minutes instead of 30 minutes, (c) if milk was heated for short time holding at 69° instead of 71° C., (d) if 0.2% raw milk was added to properly pasteurized milk.

In order to prevent bacterial action during the longer holding time, p-chlorobenzoic acid was added to reagent (a) at the rate of 5 g. per liter. Although a Pulfrich photometer was used to make the readings, the observations also can be made with the naked eye with a little practice.

I. Peters

99. **The use of ascorbic acid in controlling oxidized flavor in milk.** B. WEINSTEIN, M. LOWENSTEIN and H. C. OLSON, Okla. Agr. Expt. Sta., Stillwater. *Milk Plant Monthly*, 37, 10: 116-119. Oct., 1948.

The object of this experiment was two-fold, namely: (a) to determine how much ascorbic acid must be added to prevent the development of an oxidized flavor, and (b) how much ascorbic acid must be added to prevent the development of the off flavor in milk which contained various amounts of added copper. All determinations of ascorbic acid were made utilizing the modified Woessner method.

Milk produced in winter showed a greater tendency to become oxidized than that produced in spring. Addition of 35 mg. of ascorbic acid per l. of milk prevented development of an oxidized flavor in milk held at 45° F. for 72 hr., although identical unfortified samples became oxidized. In no case did 35 mg. of ascorbic acid prevent oxidized flavor in samples containing 0.25 p.p.m. or more of added copper. In those samples containing 0.25 p.p.m. of added copper, 50 mg. of ascorbic acid was necessary to prevent the defect and in severe cases, such as samples containing 1 p.p.m. of added copper, as much as 150 mg. of ascorbic acid was required. In all cases milk which became oxidized possessed a lower ascorbic acid content at the end of the storage period than those samples which did not become oxidized.

J. A. Meisef, Jr.

100. **Lactic acid polymers as constituents of synthetic resins and coatings.** P. D. WATSON, Agr. Research Admin., U.S.D.A., Washington, D. C. *Ind. Eng. Chem.*, 40, 8: 1393-1397. 1948.

Lactic acid is produced by fermentation of the carbohydrates present in corn sugar, molasses and whey. Although cheese and casein whey are a potential source for 400,000,000 lb. of lactic acid, only 6,000,000 lb. are produced annually from all sources. The relatively high price of the purified acid has retarded its greater use. About 400,000 lb. now are used in the plastics industry. This paper describes modified lactic acid condensation polymers which may be of interest to the coatings industry. The most useful of these products appears to be a modified poly(lactyl acid-fatty oil polymer, from which tough, water-

resistant coatings may be formulated. Another class of resins is the metal poly(lactyl lactates derived almost entirely from lactic acid; these may be used for protective and decorative coatings.

B. H. Webb

Also see abs. no. 82, 83, 107.

DAIRY ENGINEERING

A. W. FARRELL, SECTION EDITOR

101. **Vacuum treatment of milk powder.** H. SHIPSTEAD. (Assigned to Borden Co.) U. S. Patent 2,453,277. 3 claims. Nov. 9, 1948. *Official Gaz. U. S. Pat. Office*, 616, 2: 444. 1948

Milk is spray dried in the conventional manner except that the moisture content is higher than that desired in the final product. The hot powder is conveyed without loss of heat to a vacuum chamber where a portion of the moisture in the powder is flashed off and the product is cooled.

R. Whitaker

102. **Method for the evaporation and concentration of liquids.** G. G. ZAHM. (Assigned to Hurd Corp.) U. S. Patent 2,450,774. 3 claims. Oct. 5, 1948. *Official Gaz. U. S. Pat. Office*, 615, 1: 209. 1948.

Liquid food products such as milk are passed in a film over a heat exchanger under vacuum. The vapors are condensed under vacuum and the flavor containing constituents in concentrated form may be collected and returned to the concentrated solids if desired. The process is conducted rapidly at a high vacuum and relatively little heated flavor is produced.

R. Whitaker

Also see abs. no. 116, 122.

DAIRY PLANT MANAGEMENT AND ECONOMICS

103. **The future of milk consumption. A review of the main factors influencing milk consumption.** L. SPENCER, Cornell Univ., Ithaca, N. Y. *Milk Plant Monthly*, 37, 10: 124-130. Oct., 1948.

Those factors of greatest importance in influencing future milk consumption in the U. S. are: (a) population, (b) convenience, service, quality and availability of milk as compared to other foods. The author concludes that the general outlook of milk consumption is favorable, although there is a definite need for improving the attitude of the consumer towards the dairy industry. This he reasons can be done only by an increased effort to inform the public as to costs, prices and profits encountered by the industry, as well as to the nutritive value of the product.

J. A. Meiser, Jr.

104. How the Boston formula works. W. C. WELDON, H. P. Hood and Sons, Boston, Mass. *Am. Milk Rev.*, 10, 11: 32, 34, 78, 79. Nov., 1948.

A new formula for determining the price which farmers receive for class 1 or fluid milk was put into effect in the Federal order markets of Boston, Fall River and Lowell-Lawrence on April 1, 1948. The formula is based upon an average of three separate indices, (a) wholesale commodity price index for the entire United States, as prepared by the Bureau of Labor Statistics, (b) department store sales index for New England, published by Federal Reserve Bank, and (c) farm labor and feed cost index prepared by the Federal Market Administrator. The average index is applied to the base price which existed in 1925-29. Price changes occur at 22-cent intervals when called for by the formula. Two additional price stipulations were super-imposed on the above formula price. A seasonal control factor provides for an increase of 44 cents per 100 lb. in the fourth calendar quarter and 44 cents decrease in the second calendar quarter. A supply-demand control factor provides for a decrease of 44 cents per 100 lb. if a surplus greater than 41% exists for the previous 12 months, and an increase of 44 cents if the surplus is less than 33%.

The older method of arriving at milk prices by making use of market quotations for butter and skim milk powder was unsatisfactory, and price changes subject to public hearings likewise proved unsatisfactory. Acceptance of the new formula has been favorable among all groups, but the real test will come when price reductions are called for.

D. J. Hankinson

105. Laboratory control of finished dairy products. K. G. WECKEL, Univ. of Wis., Madison. *Milk Plant Monthly*, 37, 9: 34-36. Sept., 1948.

The author divides the laboratory control of dairy products into four phases: (a) chemical control, (b) bacteriological control, (c) control of physical properties, and (d) control of organoleptical properties. A brief discussion of methods used to effect these controls is presented.

J. A. Meiser, Jr.

106. Ten tips to thrift. A. C. KIECHLIN, Pompton Lakes, N. J. *Ice Cream Rev.*, 32, 5: 37, 72-74. Dec., 1948.

The author offers 10 helpful suggestions for keeping the tax expense at a minimum. The suggestions offered are as follows: (a) Deduct adequate depreciation to cover normal wear and tear on business property, machinery, non-me-

chanical equipment trucks, fixtures, etc. (b) Trade-in allowances on equipment when properly handled may result in an income tax reduction. Whenever the trade-in allowance of equipment is less than the unrecovered book value of the equipment, a tax saving will result by selling the equipment for cash and recording the loss on the books. Such losses then may be deducted on the income tax return. (c) Repair and maintenance costs should be considered as expense items which are deductible from income. Such items should not be considered as increasing the value of the assets of the business in which case they will increase the amount of tax to be paid. (d) Bad debts, both personal and business, should be written off and deducted. (e) Non-business expenses which include any items paid during the taxable year in connection with the earning of taxable income should be recorded and deducted. (f) Inventories should be carefully recorded and figured at cost or market value whichever is the lower. (g) Since deductions overlooked one year are not deductible the following year, it is important that the tax return be set up in rough form before the books for the current year are closed. The return then can be examined to make certain no transactions which might reduce the income tax have been overlooked. (h) Transactions throughout the year should be followed closely to make certain they are recorded to conform with those regulations which will give the maximum relief from taxes. (i) Record promptly and accurately all transactions. Do not trust to the memory to recall details of transactions several weeks or months old. (j) Figure the income tax early enough to make it possible to determine what effect, if any, certain adjustments might have on the tax to be paid.

W. J. Caulfield

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

107. Comparison of methods for the determination of carotene. J. V. DERBY, JR., and J. B. DEWITT, U. S. Dept. of Interior, Fish and Wildlife Service. *J. Assoc. Offic. Agr. Chemists*, 31, 4: 704-708. Nov., 1948.

Replicate samples of dehydrated alfalfa leaf meal were assayed for carotene content by using the methods of Wall and Kelley (*Ind. Eng. Chem., Anal. Ed.*, 15: 18. 1943), a modified A.O.A.C. procedure, saponification of the extracts of the modified A.O.A.C. procedure and an alcohol potash digestion method for extraction of pigments. When the extinction coefficients of the carotene solutions were plotted

against wave length, the results obtained by the 4 procedures varied. The curves indicated that the extracts differed in amounts of other carotenoid pigments. The solution obtained by the Wall and Kelley procedure appeared to contain the highest percentage of pure β -carotene, although the total content of carotenoids was lower than that of any of the other methods. The amount of carotene found by the modified A.O.A.C. procedure was 45% greater than that found by the Wall and Kelley method. Differences observed in the results of assays on replicate samples were thought to be due to varying degrees of efficacy of the extraction technics.

F. J. Babel

108. Wood yeast as a protein supplement. E. G. RITZMAN, Univ. of N. H. Guernsey Breeders' J., 74, 6: 1025-1026, 1070. Nov., 1948.

Two groups of three Guernsey cows each, were fed a grain mixture containing wood yeast as a protein supplement. Soybean meal was used in the check ration. The grain mixtures contained approximately 20% crude protein, with 234 lb. of wood yeast replacing 250 lb. of soybean meal.

Group I was fed the mixture with wood yeast for 4 weeks and then switched to the mixture with soybean meal for 4 weeks. Group II was fed in the reverse order. The wood yeast appeared to give as good results in milk production as soybean meal.

A. R. Porter

Also see abs. no. 111, 124.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

109. Your herd sire—expense or investment? G. E. GORDON, Univ. of Calif. The Jersey Bull., 67, 22: 1932-1933, 2041-2045. Nov. 25, 1948.

Cost figures from the San Pasqual Breeders Association, Inc., San Diego County, California, show \$146.53 annual costs per bull for investment and capital. Current annual costs averaged \$222.00 for feed, \$81.11 for labor and \$7.50 for veterinary service and miscellaneous, or a total annual cost of \$457.14 per bull.

A bull with 8 daughters averaging 42 lb. of butterfat more than their dams is estimated to contribute an additional \$336.00 per year to the herd income or \$1680.00 increased income over a 5-year period. In addition, his bull calves sold for higher prices and his daughters had greater sale value. Bull calves from an unproved bull sold for an average of \$168.00. After his proving with 110 lb. of butterfat increase for his daughters over their dams, his sons sold for

an average of \$455.00 each. The figures given show the need for careful selection of a herd sire as a good investment.

A. R. Porter

110. Die Leistungsvererbungen Schweizer Braunviehtierte in der Allgäuer Herdebuchzucht, unter besonderer Berücksichtigung des Fettprozentgehaltes. (The heritable factors of the Brown Swiss bulls in the Allgäu pedigree cattle breeds with special consideration of the percentage of butterfat.) English summary. H. KOPPRICH. Die Milchwissenschaft, 3, 4: 89-97. 1948.

The records of 13 domestic and imported Brown Swiss bulls were examined for herd improvement. The milk yield and the per cent butterfat of the daughters were compared with that of the respective dams of the domestic Allgäu breed. The results showed that both superior and inferior bulls had been imported from Switzerland. It was concluded that the import of expensive sires was justifiable only in cases where herd improvement was certain.

I. Peters

111. Forage crop management for higher yields. C. M. HARRISON, Mich. State College, E. Lansing. Hoards Dairymen, 93, 22: 859. Nov., 1948.

The value of the following mixtures for hay, pasture and green manure was studied: I. Red clover, alsike clover, white clover, timothy and Kentucky blue grass. II. Timothy, smooth bromegrass, perennial rye grass, Kentucky and Canada blue grass. III. Alfalfa, red clover, alsike, white clover and smooth bromegrass. IV. Alfalfa, red clover, alsike clover and white clover. Four blocks of each mixture were left for hay, 4 were pastured continuously by sheep and the remaining 4 were used for first hay and second crop pasture. The three systems of management were carried on over a 3-year period. Mixtures III and IV produced approximately twice as much hay over the 3-year period as did mixtures I and II. Mixture I (predominantly red clover the first year) produced very little more the first year or in total than did mixture II. Mixtures I and II produced no second cutting in 2 of the 3 years and a comparatively small amount only in the first year. Yields under grazing treatment varied in much the same manner as they did when cut for hay. During the 3 years, mixture I produced 9,079 lb. hay and 828 days of grazing; mixture II, 8,213 lb. hay and 774 days; mixture III, 14,703 lb. and 2,058 days; and mixture IV, 11,689 lb. and 2,022 days. It appears that mixtures containing alfalfa were almost twice as productive as the straight grass or grass-red clover mixture when measured as hay or by grazing livestock.

J. B. Frye, Jr.

ICE CREAM

C. D. DAHLE, SECTION EDITOR

112. Ice cream mixes as influenced by homogenization of only a part of the mix. W. A. KRIENKE and R. A. HIBBS, Fla. Agr. Expt. Sta., Gainesville. *Ice Cream Trade J.*, 44, 11: 48, 49, 78, 79. Nov., 1948.

Fractional homogenization of ice cream mixes made from cream (40% fat), condensed skim milk (36% serum solids), sodium alginate and water (180 p.p.m. hardness) proved satisfactory when 54% of the mix containing all the milk products was homogenized. Satisfactory whipping resulted and the body and texture score of the ice cream was only slightly below that of ice cream made from a completely homogenized mix. This practice if used would result in some saving on power and a smaller homogenizer could be used.

W. H. Martin

113. Superheating ice cream mixes. W. H. MARTIN, Dairy Husb. Dept., Kansas State College. *Ice cream Field*, 52, 4: 65-6. Oct., 1948.

The author reports results obtained on superheating ice cream mixes by injecting live steam into mix made in a vacuum pan. Heating the mix at 190° F. for 10 minutes increased mix viscosity 33%, whereas heating at 210° F. for 10 minutes increased mix viscosity 250% over that of mixes not superheated. The whipping properties of mixes were impaired slightly by superheating. Superheating mixes at 190° F. for 10 min. or at a higher temperature or for a longer time impaired the flavor of the resulting ice cream. The body and texture of ice cream made from superheated mixes containing stabilizer were equal to and often superior to ice cream manufactured from mixes not superheated. Ice cream made from superheated mixes containing no stabilizer was coarse in texture.

Bacterial destruction as a result of superheating was efficient. Counts of 200 to 400 bacteria per ml. were common, and it is claimed that the process can be depended upon to reduce the bacteria count to 10,000 per ml. or less.

W. C. Cole

114. Storing milk solids for ice cream. C. D. DAHLE, W. F. COLLINS, and J. A. MEISER, JR., Dairy Dept. Penn. State College. *Ice Cream Field*, 52, 4: 53. Oct., 1948.

The following recommendations are made:

Frozen cream: Use sweet cream with 40% fat or more, pasteurize at 170° F. for 10 minutes or longer, cool and run into new tinned 50 lb. cans. Freeze at -15° F. and store at about

-10° F. Avoid copper equipment. Employ suitable crushers or slicers to avoid mess and work of defrosting.

Plastic cream: Precautions for frozen cream apply except that the fat content is about 80%. Use essentially as butter of 93 score.

Concentrated mix: A concentrated mix or a blend of fat and solids-not-fat of the proper ratio can be frozen and stored. Heat to 170° F. for 10 minutes before condensing. Use copper free equipment.

Frozen mix: Pasteurize the whole mix at 170° F. for 20 minutes before freezing; may be stored for 6 months and does not require rehomo-genization, but authors advise storing concentrated mix instead.

Frozen condensed milk: Plain condensed skim milk tends to curdle when frozen and held for long periods of time, but the product will disperse in the hot mix if handled properly. They recommend preheating of skim milk at 160 to 170° F. instead of the higher temperatures commonly used; condense to 3:1 or 4:1 and freeze; store at -10 to -15° F. Either plain or superheated condensed skim milk may be frozen and stored, and the product has been useable after 6-12 months in storage.

W. C. Cole

115. Fried ice cream. ANONYMOUS. *Ice Cream Rev.*, 32, 5: 40. Dec., 1948.

A new novelty item recently introduced in the ice cream field is "Frigid Frys" or fried ice cream. In making this novelty a portion of ice cream of any desired shape is covered with a specially prepared dough and fried in deep fat for approximately 1 minute. This will cook the dough to a light brown color without melting the ice cream. Frigid Frys require no special dishes for serving and are well adapted for the carry out trade. They are said to have all the sales appeal of pic a la mode. It is anticipated that exclusive dealer territory will be established for distribution of this new novelty item.

W. J. Caulfield

116. Wind tunnel eliminates need for big hardening room. ANONYMOUS. *Ice Cream Rev.*, 32, 4: 74, 76. Nov., 1948.

A compact wind tunnel costing approximately \$3,000 has been installed in the plant of the Kilgore Eastland Creamery, Eastland, Texas. The tunnel with suitable shelving occupies a space 3 feet wide, 6 feet high and 8.5 feet long. It is capable of hardening the output of an 80-gallon per hr. continuous freezer in a continuous movement process.

When a container of ice cream from the freezer

is placed on a shelf on the tunnel, it is used to push ahead the container in front of it. By the time the ice cream has travelled the length of the tunnel, it is thoroughly hardened and may be stored in the hardening room or loaded directly onto trucks.

Advantages claimed for the comparatively low-cost hardening tunnel are smoother textured ice cream and less need for maintaining a large stock of ice cream on hand. W. J. Caulfield

117. Refrigerating with dry ice. G. B. ARMSTRONG, Mathieson Chemical Corp. *Ice Cream Field*, 52, 2: 31, 34, 35. Aug., 1948.

Dry ice now is being used in large trucks for wholesale delivery and small trucks for door-to-door sales, as well as in ice cream cabinets, in retail stores and to refrigerate take home packages of ice cream. In wholesale delivery trucks, bunkers are installed so they will be accessible when the truck is fully loaded. Air circulation may be helped by installing blowers. For economical use of dry ice refrigeration of trucks, (a) adequate bunker capacity, (b) use of economical design of truck body, (c) efficient insulation—at least 6 inches of standard insulation and (d) proper hardening of ice cream before loading truck are stressed. A well-constructed ice cream truck body with 3 compartments (400 gal. capacity) should not exceed an average daily requirement of 50 lb. of dry ice. A 2-compartment dry ice cabinet in a room at 70° F. requires about 11 lb. per day. An ice cream packing chart for dry ice accompanies the article.

W. C. Cole

118. Abbotts solves its truck moisture problems. E. W. HOGREN, Abbotts Dairies. *Ice Cream Field*, 52, 2: 32-33. Aug., 1948.

For somewhat over 20 years, Abbotts Dairies Fleet Maintenance Dept. has had in effect a checking system to reduce to a minimum the accumulation of moisture in truck bodies. This check includes: (a) Weighing of the truck annually to determine any increase in gross weight of empty trucks compared with these weights when the trucks were new; the cause of any increase is determined. (b) A regular inspection is made of the refrigerated plates. (c) The electrical energy required to operate the compressors is measured. (d) All refrigerated units are defrosted once a month.

An expanded polystyrene is used for insulation, with ends and sides 6 in. thick and floor and roof 8 in. thick. A water asphalt emulsion is used on the joints and on the outside of the insulation just under the metal covering.

The delivery trucks are equipped with mechanical refrigeration compressors of 1.5 H.P. capacity connected to 4 eutectic plates, while the transport trucks are refrigerated with dry ice.

W. C. Cole

119. Ice cream preference tests. P. S. LUCAS, Mich. Agr. Expt. Sta., E. Lansing. *Am. Milk Rev.*, 10, 12: 56-58. Dec., 1948.

Pure vs. imitation vanilla and strawberry flavors were rated in order of preference by the general public. Five combinations of vanilla flavoring were rated by 44 persons as follows: first—pure vanilla; second—imitation vanilla; third—pure vanilla reinforced with vanillin; fourth—pure vanilla reinforced with coumarin and fifth—vanilla fortifier only. Five combinations of strawberry flavoring were rated by a group of 15 experienced scorers as follows: first—true fruit strawberry extract; second—true fruit strawberry concentrate; third—strawberry flavor, single strength, reinforced; fourth—imitation strawberry flavor and fifth—special imitation strawberry. The above 5 strawberry flavor combinations were rated by 23 inexperienced scorers in the same order as the experienced scorers except the second and third ratings were reversed. The above strawberry flavoring materials were added to 5 gal. ice cream mix plus 3 qt. strawberries. It was concluded from this study that consumers prefer pure flavoring materials in ice cream.

D J Hankinson

120. Slide rule to greater dealer profits. T. E. HEINDENREICH, JR. *Ice Cream Trade J.*, 44, 11: 33, 71, 72. Nov., 1948.

A slide rule to be used in the determination of the exact cost of any fountain item has been developed by the General Ice Cream Co. of Schenectady, N. Y. "The working portion of the rule is a slide which can be set at the cost per gallon of ice cream. Four sets of figures come into view showing the exact cost of 5 different scoop sizes, 4 weights of pints, 5 weights of quarts and the factory filled pints. All figures are based on a 78 oz. gallon."

W. H. Martin

121. Combined spoon and lid for containers. R. C. WILSON. U. S. Patent 2,453,393. 2 claims. Nov. 9, 1948. Official Gaz. U. S. Pat. Office, 616, 2: 474. 1948.

The disk-shaped lid of a paper cup, suitable for ice cream, is scored in such a manner that a section of the lid may be easily detached and folded to form a spoon-like member.

R. Whitaker

Also see abs. no. 87, 88.

MILK

P. H. TRACY, SECTION EDITOR

122. Die Milchannahme vom Fahrzeug bis zum Annahmebehälter. (Reception of milk.) English summary. W. SCHULZ. Die Milchwissenschaft, 3, 2: 29-36. 1948.

Milk should be loaded into trucks in a manner permitting quick and easy unloading. Loading and unloading from the side is preferable. A mechanical can carrier with moving chains is best and for easy unloading should not be higher than the lowest loaded truck. Cans should pass through rinse, defroster (if necessary) and cover loosener and should be examined on their way to the dump tank. For quick, easy dumping of milk, a suitable can dumper should be installed, requiring a minimum of physical labor, leaving the cans in an inverted position on their way to the can washer. I. Peters

123. Observations on rough or "spotty" homogenized milk. G. M. TROUT and J. R. BRUNNER, Mich. State College, E. Lansing. Milk Plant Monthly, 37, 10: 92-94. Oct., 1948.

The rough granular substance adhering to the inner surface of a partially emptied homogenized milk bottle was found by microscopic examination to be a fatty material that was stained readily by Sudan III. Routine line checks of the processing operation proved that non-homogenized milk was contaminating the homogenized product in the bottle filler reservoirs. Either ceasing to bottle cream prior to bottling homogenized milk or a thorough clean-up between operations eliminated this defect. J. A. Meiser, Jr.

124. Benzene hexachloride flavored milk. H. G. LINDQUIST and R. W. DONALDSON, Univ. of Mass., Amherst. J. Milk and Food Technol., 11, 6: 325-326. Nov.-Dec., 1948.

Potatoes grown in fields treated with benzene hexachloride, or 666 to destroy wire worms, were rendered unfit for human consumption. A utilization of these potatoes as cattle feed prompted the authors to study the effects on the flavor of milk from cows fed these products. An off-flavor was observed in the potatoes and in the milk. However, the flavor varies in intensity and only a keen sense of taste will detect it. No specific chemical test was satisfactory to detect the presence of this compound in milk. The investigators are of the opinion that the presence of small amounts of benzene hexachloride in potatoes or in milk obtained from cows fed the potatoes is harmless to humans. H. H. Weiser

125. Paper bottle top. J. P. JONES. (Assigned to Dairy Specialties, Inc.) U. S. Patent 2,453,133. 2 claims. Nov. 9, 1948. Official Gaz. U. S. Pat. Office, 616, 2: 408. 1948.

Milk may be poured easily from a paper bottle by inserting this device which consists of a gradually pointed tube, ending in a discharge spout and provided with a projection to determine extent of insertion. R. Whitaker

126. Milk delivery tube. J. A. HOPWOOD. (Assigned to Monitor Process Corp.) U. S. Patent 2,449,229. 2 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 455. 1948.

A tube for draining milk from milk cans is described; it may be detached easily for cleaning. R. Whitaker

127. The reseparation method for increasing the viscosity of cream. A. C. SMITH and F. J. DOAN, Penn. Agr. Expt. Sta., State College. Milk Plant Monthly, 37, 10: 84-88. Oct., 1948.

Using a variable speed separator and maintaining accurate temperature controls, the authors found that the increase in viscosity obtained by the reseparation method was not due to centrifugal action, but was the result of a controlled heating and cooling treatment. This treatment, they reasoned, was nothing more than a variation of Hening and Dahlberg's rebodifying process. The reseparation method offered several disadvantages in that it enhanced cream plug formation and serum separation in aged cream and necessitated the use of an extra piece of equipment.

J. A. Meiser, Jr.

128. Cream defects—their causes and preventions. LYNN R. GLAZIER, Pfaunder Co., Rochester, N. Y. Am. Milk Rev., 10, 12: 64-65. Dec., 1948.

The 10 defects described are cream plug, feathering, foaming, high acidity, lipase activation, off-flavors, oiling-off, poor whipping ability, poor viscosity and serum separation. Causes and preventions are listed in tabular form.

D. J. Hankinson

129. Milk fat in milk chocolate. L. W. FERRIS, Food and Drug Admin., Federal Security Agency, Buffalo, N. Y. J. Assoc. Offic. Agr. Chemists, 31, 4: 728-731. Nov., 1948.

Results are presented which show that straight ether extraction of milk chocolate leaves an appreciable amount of milk fat unextracted. A method is presented which effects a more complete extraction of the fat. The method consists of extraction with ether, followed by the addition

of 1,4-dioxane and heating and final extraction with ether. In one instance slightly more than 1% of additional milk fat was obtained by the proposed method.

F. J. Babel

130. Milk vending—a new horizon. E. J. Newcomer, City Milk Co., Inc. *Am. Milk Rev.*, 10, 11: 40, 42. Nov., 1948.

Vending machines have not been used widely for dispensing milk. Health regulations and the wide variety of bottles used for milk have discouraged the design of automatic milk vending machines. However, automatic equipment now is available to dispense all types of containers, and some machines will dispense both chocolate and plain milk. When two products are delivered by the same machine, it is possible to serve as many as 168 persons in 15 minutes. Automatic vending equipment is compared with manually operated equipment, which served only 35 units in 15 minutes. Automatic vending equipment is considered most adaptable to factory service and apartment houses. Machines must be capable of accepting varying amounts of money, because the price of milk varies widely in different areas. Either the 1/3 qt. or 10-oz. bottle is recommended. It is pointed out that consumers readily choose milk instead of other beverages where both milk and beverage vending machines are in use.

D. J. Hankinson

131. Concentrated apple juice and milk modifier. H. St. Clair. (Assigned to Ridgewood, Inc.) U. S. Patent 2,450,456. 10 claims. Oct. 5, 1948. *Official Gaz. U. S. Pat. Office*, 615, 1: 133. 1948.

Milk for feeding infants and children is made more digestible and nutritious by blending with concentrated apple juice having a pH of 4.5 to 5.5 and a density of 68 to 75° Brix.

R. Whitaker

Also see abs. no. 89, 93, 97, 98, 99, 103, 104, 133.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

132. Die Regulierung der Laktation. (The regulation of the lactation.) English summary. E. FAUVET. *Die Milchwissenschaft*, 3, 3: 61–65. 1948.

Lactation is a nutritive process found in mammals as well as in some lower classes of animals. In the lower classes of animals, some males and gonad-less individuals are capable of nursing the young. During the process of evolution, lactation has become an economic and purely maternal nutritive performance.

In the higher mammals the rudimentary mammary glands develop during pregnancy and start functioning autonomically after the disappearance of progesterone. The pituitary gland takes no active part in this process at first, since the hypothetically assumed milk-forming hormone does not exist. Only through the stimulatory act of sucking is the pituitary gland caused to produce a histotrope hormone, the latter maintaining and renewing continuously the parenchyma of the lactative glands. By means of this change, the mammary gland becomes independent of its follicle hormone and becomes subordinate to the pituitary controlling growth factor. I. Peters

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

133. The fortification of milk with vitamins A and D. Council on Foods and Nutrition. *J. Am. Med. Assoc.*, 138, 1: 23. Sept. 4, 1948.

The Council long has approved fortification of milk with vitamin D, but it has stated the opinion that fortification of milk as a general purpose food with vitamin is not in the interest of public health. There is no objection to the presence of vitamin A when the preparation used for fortifying with vitamin D contains vitamin A in natural association. Fish liver concentrates often contain large amounts of vitamin A, and milk fortified with these concentrates has been accepted and a statement of vitamin A content has been permitted on the label.

The Council believes that, even though the procedure apparently is harmless, evidence is lacking that fortification with vitamin A is in the interest of public health and does not constitute reason for the acceptance of advertising that suggests special nutritional advantages. Since milk is a good source of vitamin A, additional fortification with vitamin A may add to the cost of the milk.

D. P. Glick

SANITATION AND CLEANSING

K G. WECKEL, SECTION EDITOR

134. Wetting Agents. Some facts about the nature, uses and economic importance of wetting agents. R. H. Smith, E. I. Du Pont de Nemours & Co., Chicago, Ill. *Milk Plant Monthly*, 37, 9: 80–81. Sept., 1948.

Wetting agents may be classified according to: (a) the source of the raw material used in the manufacture of the compound, (b) the type ion produced when the compound is dissolved in water, and (c) the physical properties of the compound.

The vast majority of wetting agents belong to the anionic group, being produced from non-fat raw materials, and should possess one or more of the following properties: (a) sequestering action, (b) power of penetration, (c) power of suspension, (d) power of dispersion, (e) emulsifying ability, (f) detergency, and (g) ability to lower surface tension.

Considering the above properties, all detergents may be called wetting agents. However, not all wetting agents can be classified as detergents.

J. A. Meiser, Jr.

135. Let's look at brushes. NORMAN MYRICK. *Am. Milk Rev.*, 10, 12: 34-35. Dec., 1948.

Since 20 to 25% of the labor in a milk plant is expended for cleaning, a knowledge of brushes is important. The diameter of a brush for the inside of sanitary pipe should be greater than the diameter of the pipe, but not so much greater that matting of the brushes occurs and swabbing action instead of brushing action takes place. The bristle should bow somewhat, allowing the end to do the cleaning. Separator discs are cleaned between 2 counter-revolving brushes. Solution-fed brushes are useful for cleaning large surfaces. Nylon bristles, although more expensive than animal fibre bristle brushes, are more durable. Furthermore, nylon bristles are available in various diameters and do not become water-soaked. Motor driven brushes also are useful. Cleaning solution temperature of 115° F. is suggested.

D. J. Hankinson

136. The influence of pH on the efficiency of hypochlorite solutions in sterilizing metal surfaces. C. M. COUSINS and J. WOLF, Milton Antiseptic Ltd., and Deosan Ltd., John Milton House, Brewery Rd., London, N. 7. *Proc. Soc. Applied Bact.*, 1946, 1: 15-19. 1946.

Experiments were conducted with a culture of *Staph. aureus* and 2 cultures of thermophilic micrococci which were suspended in 10% skim milk, then inoculated on metal trays and dried. The highest destruction of the bacteria by hypochlorites containing 25, 50, 100 and 200 p.p.m. available chlorine were at the alkaline pH values of 9.5, 10, 10.7 and 11, respectively. Spores of *B. subtilis*, however, were destroyed by hypochlorites (50 p.p.m.) more quickly at pH 7 than at higher pH values. This difference in activity for hypochlorites on dried vegetative cells and dried spores was believed to be caused by formation of a chloroprotein in the case of the vegetative cells which have a cell wall of protein nature exposed to the action of dissociated hypochlorite. The refractile walls of spores were considered not to be of this protein nature, their destruction being dependent

on the concentration of undissociated hypochlorite present.

M. L. Speck

137. Sterilization of dishes and utensils in eating establishments. G. R. WEBER, Milk and Food Sanitation Lab., U. S. Public Health Service, Cincinnati, Ohio. *J. Milk and Food Technol.*, 11, 6: 327-333, 351. Nov.-Dec., 1948.

Sanitization of dishes and utensils can be accomplished by cleaning all utensils with an acceptable detergent and rinsed, with hot water at 170° F. for at least 2 min., with chlorine, or quaternary ammonium compounds. Since the latter compounds are not used widely, the author believes that they should be tested carefully before being put into practical use. The type, number of organisms and composition of the water used are important factors in the sanitization of eating utensils.

H. H. Weiser

138. Good housekeeping, an essential of modern dairy plant operation. W. M. ROBERTS, Dairy Manufacturing Dept., North Carolina. *Milk Plant Monthly*, 37, 10: 102-104. Oct., 1948.

Good housekeeping in the dairy plant insures a high quality product that is more economical to produce. It provides sound advertising and prevents accidents in the plant that may lower the morale of the worker.

Certain questions that may be used in evaluating your plant from the good housekeeping standpoint are: (a) Are the outside surroundings of the plant neat and attractive? (b) Does the appearance of the manager's office set the standard for the plant? (c) Are the laboratories spotless and well arranged? (d) Are the processing rooms clean and devoid of leaky fittings? (e) Are adequate storage facilities provided, and are they kept clean? (f) Are locker rooms and toilets maintained in an orderly and sanitary manner? (g) Does the engine room meet the standards of the remainder of the plant?

It is the responsibility of the plant owner or manager to insure good housekeeping in the plant, and it is his duty to direct, instruct and provide facilities for maintaining good housekeeping in the plant.

J. A. Meiser, Jr.

139. Clip for cleaner milk. N. N. ALLEN, Univ. of Wis., Madison. *Hoards Dairyman*, 93, 22: 851. Nov., 1948.

In this study every other cow in the milking line had the udder, flanks, thighs and tail clipped in the manner generally recommended. Alternate cows were unclipped. The cows were milked by hand and machine during the experiment. Samples for bacteria and sediment tests were taken before the milk was strained. The

bacteria count of the milk produced by the clipped cows definitely was lower than that of the unclipped cows when the cows were hand milked. When the cows were machine milked, the averages were in favor of the clipped group. Clipping did not reduce the sediment in the milk enough to change the grade. However, it is pointed out that the sediment test is not a very exact one and will measure only marked differences.

J. B. Frye, Jr.

140. Types of material in accumulated dust and fine debris. T. J. CLAYDON, Kansas Agr. Expt. Sta., Manhattan. *Am. Milk Rev.*, 10, 11: 24, 76, 77. Nov., 1948.

The dust and debris accumulations on ledges in creameries, cream stations and farms are shown to be closely related to the type of sediment found

in empty shipping cans and in sediment tests of cream. Microscopic examinations of 80 samples of fine material collected at 3 creameries, 40 cream stations and 6 farms were made. The material found was classified into one of ten types. The occurrence of the various types of material in the 80 samples was as follows: vegetable parts, 97.5%; sand, 92.5%; coal dust, 82.5%; fibers, 76.2%; rodent-like hairs, 53.7%; other hairs, 67.5%; feather parts, 50.0%; insect parts, 38.7%; metal parts, 33.7%. Material not classified in one of the above types was listed as unidentified.

Rodent-like hairs occurred most frequently in the farm samples. Insect parts occurred least in cream station samples. Coal dust and fibers occurred least in farm samples. The importance of controlling airborne contamination of cream and cream cans is indicated. D. J. Hankinson

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEW

141. **Annual review of microbiology, Vol. II.** Edited by C. E. CLIFTON. Annual Reviews, Inc., Stanford, Calif. 532 pp. \$6.00. 1948.

Microbiologists and those who are concerned with the relationships of microbiology to other fields will welcome the second volume of this series of publications. The eighteen reviews cover the wide range of topics which one has come to expect in the Annual Reviews series. The discussions are presented well, the citations to the literature are numerous and the volume is well-prepared and well-indexed.

The reviews cover the following topics: Yeasts, Genetics of the Fungi, Bacterial Metabolism, the Metabolism of Malarial Parasites, Growth Factors for Microorganisms, Antibiotics, The Mode of Action of Chemotherapeutic Agents, Inheritance of Immunity in Animals, Complement, The Nature of Antibodies, Pathogenic Streptococci, the Spirochetes, The Neurotropic Viruses, Bacteria as Plant Pathogens, Chemical Disinfectants, Microbiology of Drinking Water, and Sewage, Microbiology of Soil and Biological Nitrogen Fixation.

F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

142. **Vibrio foetus infection in cattle.** A. S. CANHAM, Allerton Laboratory, Pietermaritzburg, South Africa. J. S. African Vet. Med. Assoc., 19, 3: 103. Sept., 1948.

Several case histories of *Vibrio foetus* infection in cattle are reported. The disease is not contagious, therefore does not appear to be as serious as contagious abortion. Cows aborted from 4 to 7 mo. after pregnancy and in all cases retained placenta were observed. All cows were isolated until cleaned and treated with daily irrigation of saline. When the animals appeared normal they were bred. Normal pregnancies and births of living calves followed in all cases. The disease is widespread in South Africa. However, in no herds has there been a complete loss of a calf crop and few cows fail to recover and breed normally after their first abortion.

K. M. Dunn

143. **The diagnosis of east coast fever in South Africa.** A. M. DIESEL AND G. C. VAN DRIMMELEN. Institute of Onderstepoort, Pretoria, South Africa. J. S. African Vet. Med. Assoc., 19, 3: 81. Sept., 1948.

A review of the disease, covering its history and methods of diagnosis, is presented. The authors point out the similarity of this disease to those caused by protozoal diseases such as *T. parva* and anaplasmosis. The present methods of diagnosis are based on both epizootological and protozoological studies. Detection of the parasites is a difficult job and requires well-trained personnel both in collecting samples and for microscopic examinations.

K. M. Dunn

144. **Treponema theileri of a donkey and its transmission to a calf.** V. R. KASCHULA, Pietermaritzburg, South Africa. J. S. African Vet. Med. Assoc., 19, 3: 100. Sept., 1948.

A successful attempt to transmit *Treponema theileri* from the donkey to a calf by blood transfusion is reported. It required 18 d. for the calf to show fever and for *T. theileri* to appear in the blood as detected from smears. No rough or scaly skin was observed in the calf.

K. M. Dunn

BUTTER

O. F. HUNZIKER, SECTION EDITOR

145. **Das physikalische Bild der Butter.** (The physical aspect of butter). English summary. N. KING AND W. FRITZ. Die Milchwissenschaft 3, 3: 75-82. 1948.

The continuous phase in butter consists of free, non-spherical fat. The free fat is made up of (a) mechanically broken fat globules and (b) butter oil formed in low-cooled cream. In cooling cream, the crystallizing fat migrates to the outer part of the fat globule, forming a solid circle which on prolonged cooling exerts pressure upon the inner liquid portion resulting in rupture of the globule and the liberation of the butter oil. The butter oil then forms crescent-shaped layers around the solid fat portion.

The % free fat is highest in machine butter (62%) and consists chiefly of broken fat globules. In churn butter the free fat amounts to 48% and

is made up of broken fat globules and butter oil. In Alfa butter the free fat (12%) consists mostly of butter oil. The % free fat in butter is reduced by (a) low churning temperature and (b) use of large churns. The consistency of butter is determined by (a) composition of free fat and (b) size and number of fat crystals. High butter oil and few small crystals result in sticky butter, whereas low butter oil and many large crystals result in crumbly butter. Deep cooling of winter cream prevents crumbliness, whereas in summer cream it may result in sticky butter. "Oily" butter results from churning low-cooled high-fat cream. I. Peters

146. *Das physikalische Bild der Butter.* (The physical aspect of butter). N. KING AND W. FERTZ. *Die Milchwissenschaft*, 3, 4:102-107. April, 1948.

Whereas the free fat phase plays an important role in the consistency of the butter, the aqueous phase determines primarily the initial and keeping quality of the butter, with some possible influence upon its consistency. The buttermilk droplets with a diam. below 15 μ constitute the major part of the moisture in butter, with the droplets of added water, 100 to 1,200 μ in diam., making up the rest. The washing of butter granules neither dilutes the buttermilk droplets nor decreases their size. The average size of the water droplets decreases in the following order of manufacturing procedures: butterworker \rightarrow machine butter \rightarrow Alfa butter. The size of the water droplets decreases in machine butter with (a) increasing output per hr. (79 to 470 kg. per hr.) and (b) lowering of churning temperature (11.5 to 5.8° C.) The finer distribution of water droplets improves the texture and assures greater keeping quality of the finished butter. I. Pétters

147. *Butter cutter.* E. A. ANDERSON. U. S. Patent 2,454,421. 4 claims. Nov. 23, 1948. Official Gaz. U. S. Pat. Office, 616, 4: 1016. 1948.

Butter is cut into prints by wire frames which are forced down and across through the block as it is supported on a platform. R. Whitaker

Also see abs. no. 155, 160, 162.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

148. *Comparisons of the resazurin test with other tests for the quality of milk.* J. C. BOVD AND H. C. HANSEN, Univ. of Idaho, Moscow. *Milk Dealer*, 38, 1: 200-208. Oct., 1948.

The results of a comparison, on the same milk

samples, of freshly-made solutions of methylene blue and resazurin with those 2, 3, 4 and 5 d. old do not indicate any changes in the solutions that would affect the grading of milk. A comparison of the liquid resazurin with the dry resazurin showed a correlation of almost 100%. Of 144 samples graded A by the resazurin test, 79% had a standard plate count (SPC) of < 100,000/ml. and of 236 samples graded A by the methylene blue, 71% had a SPC of < 100,000/ml.; 2.7% of those graded A by the resazurin test and 3.7% by the methylene blue had SPC's > 500,000/ml. The resazurin test placed more samples having a SPC of < 100,000/ml. in B and C grades than the methylene blue, and of the D grades the number of samples was insufficient, but the resazurin test had a tendency to place in D grade a few samples with SPC's of < 100,000/ml.

A comparison of the resazurin test and the direct microscopic count (DMC) on 408 samples showed that 65.2% of the 205 samples graded A by the 1-hr. resazurin test had a DMC of < 1 million/ml. Also 6.3% had a DMC > 5 million/ml. Of the samples graded D, 8% had a DMC of < 1 million/ml. The results of a comparison of the 1-hr. resazurin test with the 5.5-hr. methylene blue test show that the 1-hr. resazurin test will place fewer samples in A grade and more samples in D grade than will the methylene blue test. A comparison of the 1-hr. resazurin test and the alcohol test showed no correlation. A comparison of the 1-hr. resazurin test to titratable acidity showed that the samples placed in D grade by the resazurin test had titratable acidities varying from 0.16 to 0.26%, but no sample placed in A grade had a titratable acidity > 0.2%. A comparison of the 1-hr. resazurin test with the sediment test showed 14.3% of the samples graded no. 4 by sediment and 58.5% graded D by the resazurin test.

C. J. Babcock

149. *Die Verwendung der Milchzucker-Fuchsin-Papier-Platte für den Nachweis von Bact. Coli und die Bedeutung des Coliquotienten in der Milch.* (The use of lactose fuchsin-paper disk evidence of *Bact. coli* and the significance of the coli quotient in milk.) English summary. G. MÜLLER. *Die Milchwissenschaft*, 3, 3: 82-83. 1948.

The use of membrane filters in removing bacteria from water supplies and the successful growth of these organisms by placing the membrane upon an endo agar medium induced the author to adapt this method for the examination of milk. The agar medium was replaced by a paper disk saturated with a suitable nutrient, such as lactose-fuchsin; this resulted in a considerable

saving of medium. Typical coliform colonies, 2-4 mm. in diam., were obtained after 20 hr. incubation at 37° C. The method also could be used to obtain total counts in milk. The ratio of coliform count to total count (coliform \div total count \times 100) was expressed as the coliform quotient and should not exceed 0.02% in grade A milk. Samples of pasteurized milk examined showed quotients up to 10%, indicating recontamination of milk or prolonged storage at improper temperatures.

I. Peters

150. Control of thermophilic bacteria in milk. Ann. Rpt. N. Y. State Assoc. Milk Sanit., 21: 53-70. 1948.

From the standpoint of the health department. P. Corash, N. Y. City Dept. Health. Pp. 53-56. The thermophilic bacteria problems developed with the adoption of tryptone-glucose-milk agar for plating, as these bacteria grow on this medium. Although thermophilic bacteria are found in the cow's udder, it is doubtful if this source is very important. Thermophilic bacteria are not pathogenic but good sanitation requires that their numbers be low. Our research has shown no growth of thermophiles in holding tanks under proper refrigeration, but homogenization gave increased counts from 3.5 to 34% in one series of tests and an average increase of 64% in another series of experiments. Raw milk must be pasteurized down to 20,000 per ml. to be acceptable.

From the standpoint of the plant laboratory. P. E. Carney, Sheffield Farms, Inc., New York, N. Y. Pp. 57-58. Stress was given to the correct sampling procedure for milk for bacterial counts. With the increased use of high temperature pasteurization in plants, the same method might be used in the laboratory. The oval tube method of determining thermophilic bacteria was recommended to save time, agar, incubator space, and glassware.

From the standpoint of the dairy serviceman. D. T. Baker, Dairymen's League Coop. Assoc., Poughkeepsie, N. Y. Pp. 59-61. In looking for the source of high thermophilic counts, the milk cans should be given first attention. Possible causes on the farm are (a) dust contamination, (b) dirty cows, (c) filth and moisture in air lines, (d) casein in milking utensils, (e) cracked or porous rubbers, (f) cracks, rust, or dents in metal parts and (g) milk cooler not properly functioning. Suggestions were offered for correction of faulty conditions on the farm.

From the standpoint of the milking machine manufacturer. L. E. Bober, Babson Bros. Co., Chicago, Ill. Pp. 61-66. Unfortunately for the solution of the thermophilic problem, there has never been close agreement within the industry and the colleges as to what constitutes good milk

machine sanitation. In our survey of 1944-1945 involving 46 farms, 8 of the 10 farms with the highest quality rating scrubbed every part of their milkers at least once a day; of 120 milk samples taken only 2 had thermophilic counts over 15,000 per ml. The other group of 36 farms flushed their milkers. Out of 120 samples of their milk, 69 gave thermophilic counts over 30,000 per ml. It should be apparent that machines should be scrubbed clean.

From the standpoint of the milking machine manufacturer. G. H. Hopson, DeLaval Separator Co., New York, N. Y., Pp. 67-70. The personnel caring for the milking machines is extremely important in obtaining clean machines. Some dairymen always desire and try to keep equipment and product clean, some will cooperate when told and shown, but others are chronic offenders. It would appear that an incentive to produce clean milk based upon premium for quality would be very helpful.

A. C. Dahlberg

151. Thermophilic microflora of dairy utensils. S. B. THOMAS, F. JONES-EVANS, L. B. JONES AND B. F. THOMAS, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., 1946, 1: 51-53. 1946.

Farm milking utensils were examined by a rinse technic after sterilization by various methods. In order of bactericidal effectiveness, steam was best, followed by hypochlorite treatment, while washing with warm water was the poorest. Laboratory pasteurization of the rinses showed that the micro bacteria, micrococci, and spore-forming rods were the predominant types found after steam and hypochlorite treatments. Bacteria in pasteurized rinses from utensils washed with warm water were 98% microbacteria. Scrapings of milk-stone from certain utensils showed the presence of micrococci, microbacteria, spore-forming rods and streptococci in much smaller numbers.

M. L. Speck

152. Further studies of thermophilic bacteria in milk. D. A. MCKENZIE, M. MORRISON AND J. LAMBERT. Dept. of Agr., Univ. of Leeds. Proc. Soc. Applied Bact., 1946, 1: 37-39. 1946.

This study was to determine the types of thermophilic bacteria present in farm milk cans, which were found to be a very important source of such bacteria in raw milk. Counts were made on laboratory-pasteurized can rinses and plates were incubated at 30° C. The types found to be present in a total of 654 statistically picked colonies were: microbacteria, 73.1%; micrococci, 10.2%; streptococci, 2.7%; miscellaneous (gram-negative and filamentous rods), 13.9%.

M. L. Speck

153. Heat resistant bacteria in farm water supplies. S. B. THOMAS AND M. ROBERTS, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., 1946, 1: 44-46. 1946.

A series of 116 farm water supplies was examined for thermophilic and thermophilic bacteria. Thermophilic counts exceeding 100 per ml. were found in 21% of the samples and none was free of these bacteria. Classification of 342 thermophilic colonies selected at random showed the presence of the following: bacilli, 74%; micrococci, 9.1%; actinomycetes, 8.2%; gram-negative rods, 3.8%; yeasts, 2.6%; microbacteria, 2.0% and streptococci, 0.3%. Thermophiles were present in 21% of the samples, but only 2.5% contained more than 10 per ml. M. L. Speck

154. Psychrophilic bacteria in raw and commercially pasteurized milk. S. B. THOMAS AND C. V. CHANDRA SEKAR, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., 1946, 1: 47-50. 1946.

Incubation at 3-5° C. required 21 d. to give maximum counts of psychrophiles on yeastrel agar. Raw milk and pasteurized milk both contained psychrophiles, although raw milk laboratory pasteurized at 63° C. for 30 min. showed no survival of these bacteria. Also, none of the pure cultures isolated from raw milk survived this pasteurization. Of 203 psychrophiles isolated from raw and pasteurized milk, 98% were gram-negative rods and the remainder were yellow micrococci. M. L. Speck

155. Studies on starters. V. Effect of pathological or physiologically abnormal milk on acid production by lactic acid streptococci. E. B. RICE, Dairy Division, Dept. of Agr. and Stock, Brisbane, Queensland. Dairy Ind., 8, 10: 983. Oct., 1948.

The effect of mastitic milk on acid production by lactic acid streptococci differed to quite an appreciable extent in different experiments. More frequently acid formation appeared to be depressed, but in some samples acid developed to the same extent in the normal and in the mastitic milk. Even in the milk after pasteurization slower growth occurred in some samples, showing that the milk itself, and not the growth of the organisms which are heat labile, was responsible.

Development of the lactic streptococci in late lactation milk was appreciably slower than in milk from cows in full lactation. This is attributed in some measure to the high leucocyte content of late lactation milk.

Colostrum itself and milk to which it is added at the rate of 10% or more promotes the acid forming capacity of starter streptococci when in-

cubated at a constant temperature. This probably is associated with the high buffer value of colostrum. G. H. Watrous

156. The lactic acid-proteolytic bacteria and the genotypicity of the bacterial enzymes. CON-
STANTINO GORINI. Enzymologia, 12, 2: 82-87. April, 1947.

The characteristics of acido-proteolytic bacteria and especially the variations in their enzyme systems are reviewed. The possibility that enzyme variation patterns may aid in the classification of these organisms is advanced. F. E. Nelson

157. The activity of certain cationic germicides. G. J. HUCKER, R. F. BROOKS, DOROTHEAE METCALF AND WM. VAN ESELTINE. N. Y. Agr. Expt. Sta., Geneva. Food Tech., 1, 3: 321-344. July, 1947.

The germicidal action of 13 compounds was investigated against *Escherichia coli*, capsulated and non-capsulated strains of *Aerobacter aerogenes*, *Micrococcus aureus*, *Streptococcus cremoris*, *Bacillus subtilis* and three spore-forming flat sour organisms. One ml. of the germicide solution of 10 x the final concentration desired was added to 9 ml. of broth containing approximately 500 million organisms per ml., then the time required for complete killing was determined. A wide variation existed in the relative germicidal efficiency of the cationic germicides studied. The individual organisms showed considerable variation in resistance to the cationics. In general, *E. coli* was more resistant than *A. aerogenes*, while the non-capsulated strain of *A. aerogenes* usually was much less resistant than the capsulated strain of this organism. The cationic germicides were effective in killing bacterial spores, but a much greater concentration was required than for killing vegetative cells. The detergents studied showed no corrosive action on the metals commonly used in foods and dairy processing plants. E. R. Garrison

158. A study of the germicidal value of glycols, glycol benzoates and related compounds. H. C. HEIM AND C. F. POE, Univ. of Colorado, Boulder. Food Tech., 2, 1: 23. Jan., 1948.

Ethylene glycol, diethylene glycol, propylene glycol and trimethylene glycol gave phenol coefficients that varied from 0.0 to 0.021, while the six benzoic acids studied showed phenol coefficients that ranged from 0.0 to 7.8 when tested against *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) and *Eberthella typhi* (*Salmonella typhosa*). The bactericidal action of 46 derivatives of benzoic acids towards *Escherichia coli* and *Aerobacter aerogenes* was studied,

Enough of each chemical was added to lactose broth to produce saturation, then a fermentation tube of the saturated broth was inoculated with the test organism. The tubes were incubated at 37° C. and examined for growth and gas production after 12, 24, 48 and 72 hr. Most of the compounds studied failed to show any marked bactericidal properties, but the benzoates and salicylates of methyl and ethyl alcohols were very good inhibitors.

E. R. Garrison

159. A technique for studying resistance of bacterial spores to temperatures in the higher range. C. R. STRUMBO, Food Machinery Corp., San Jose, Calif. Food Tech., 2, 3: 228-240. July, 1948.

A heat exchanger (thermo-resistometer) was designed and used to determine the thermal resistance of bacterial spores. The errors due to temperature lag largely were eliminated by rapidly heating the inoculated medium to process temperature and subsequently cooling rapidly to a non-lethal temperature.

Approximately 0.01 ml. of food and 0.01 ml. of spore suspension were mixed together in a small tin cup, the sample cup was placed in an aluminum boat and both cup and boat were exposed in a steam-heated chamber for the time desired. At the end of the prescribed time, the chamber was automatically exhausted by means of an electric clock with two micro switches and the boat, cup and sample were drawn into a tube of culture medium. The culture containing the heated spores then was covered with a layer of sterile vaseline-paraffin mixture and incubated at a suitable temperature to determine sterility. The temperature of the heating chamber could be controlled to within 0.3° F. of the temperature desired and the heating time could be reproduced with an accuracy of ± 0.001 minute.

The method was developed for accurately determining thermal resistance of bacterial spores to temperatures of 240 to 270° F. but it also is applicable for use at any temperature between 215 and 270° F. A trained technician can process as many as 216 samples in an 8-hr. day by this procedure.

E. R. Garrison

Also see abs. no. 141.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

160. Apparatus for breaking and making emulsions. C. E. NORTH AND A. P. NORTH. U. S. Patent 2,455,945. 9 claims. Dec. 14, 1948. Official Gaz. U. S. Pat. Office, 617, 2: 433. 1948.

Cream can be converted into butter, and milk

and cream can be reconstituted from milk fat and dry non-fat milk solids by passing through this equipment. Both processes are accomplished continuously in castings in which specially designed agitators rotate. Emulsions are broken by the use of dished disc-shaped blades, while emulsions are formed by the use of perforated paddle-shaped agitators.

R. Whitaker

161. Cream separator and homogenizer. J. B. McFADDEN. (assigned to United Dairy Equipment Co.). U. S. Patent 2,453,924. 5 claims. Nov. 16, 1948. Official Gaz. U. S. Pat. Office, 616, 3: 740. 1948.

In a combination separator-homogenizer, whole milk is separated by centrifugal force into skim milk which is immediately discharged and cream which is homogenized by forcing it against vertical surfaces on overlapping rotating blades.

R. Whitaker

162. Centrifugal separator. H. O. VOGEL (assigned to International Harvester Co.). U. S. Patent 2,456,347. 9 claims. Dec. 14, 1948. Official Gaz. U. S. Pat. Office, 617, 2: 535. 1948.

A self-washing cream separator bowl is described. When it is desired to clean the bowl, the washing liquid is admitted to the bowl and directed through the disks by a distributor tube in such a manner that all surfaces are flushed by the cleaning fluid. The side walls of the bowl slope toward a series of outlets or ports through which the cleaning liquid escapes, carrying the milk and slime residues. The ports are open at reduced speeds and closed by means of valves which are operated by the centrifugal force at normal bowl speeds.

R. Whitaker

163. Methods used by the dairy industry to improve sanitary control through equipment design. Dairy products and liquid foods. E. H. PARFITT, Sanitary Standards Subcommittee of the Dairy Industry Committee, Chicago, Ill. Food Tech., 2, 1: 39-44. Jan., 1948.

The development of the dairy industry's interest in equipment design is reviewed. The sanitary standards approved for equipment used in all branches of the dairy industry are now arrived at by the cooperative action of three committees appointed by the Dairy Industry Committee, the International Assoc. of Milk Sanitarians and the U. S. Public Health Service. These three agencies by joint action are endeavoring to standardize the design of dairy equipment, thereby making parts uniform and interchangeable and increasing the ease with

which the equipment can be cleaned, drained and inspected. The work accomplished to date includes the development of sanitary standards for 37 fittings for milk pipes and sanitary standards for milk storage tanks, milk pumps, weigh cans and drop tanks for raw milk and homogenizers. The work in progress includes the development of standards for milk transportation, tanks, milk pails and strainers, milking machines, electric motors, can washers and heat exchangers.

E. R. Garrison

164. 100% Freon plant. ANONYMOUS. *Ice Cream Rev.*, 32, 6: 32, 67. Jan., 1949.

The Euclid-Race Ice Cream Co. in Euclid, Ohio, is the first ice cream plant in the United States using Freon-12 exclusively as the refrigerant. The plant is equipped with two 50 h.p. and two 30 h.p. compressors. These supply refrigeration for the operation of two 150-gal./hr. continuous freezers, a 24-mold brine tank rated at 176 doz. novelties per hr., two 500-gal. mix storage vats, a sweet water cooler, a hardening room maintained at -20° F. with a capacity of 55,000 gal. of ice cream and a refrigerated storage room for the storage of flavors and raw materials.

Advantages claimed for the use of Freon-12 over ammonia include: (a) greater safety, (b) saving in the amount of space required for the compressors, (c) power economy, (d) maintenance economy through the use of compact equipment and non-corrosive copper lines and (e) water economy.

W. J. Caulfield

165. Heating rates of food in glass and other containers. D. G. MERRILL. *Hartford-Empire Co.*, Hartford, Conn. *Ind. Eng. Chem.*, 40, 12: 2263-2269. Dec., 1948.

An empirical formula of general application is given for the approximate heating rate of cylinders. Data from confirming experiments are given. Constants are derived for application to the heating of food in glass containers so that the established methods for computing sterilization process time in tin may be extended to glass.

B. H. Webb

166. Use and care of water softeners. ESKEL NORDELL. The Permutit Co., New York, N. Y. *Ann. Rpt. N. Y. State Assoc. Milk Sanit.* 21: 111-121. 1948.

The hardness of dairy supply water is very objectionable because it tends to form scale and sludge when heated or concentrated, or upon addition of alkaline washing compounds. Scale is unsightly and difficult to sterilize. Insoluble granulated zeolites soften water by the "base ex-

change" method. Calcium magnesium, and ferrous iron are removed and replaced by sodium ions. After the zeolite has removed the maximum capacity of hardness, it is necessary to automatically or manually regenerate the system by backwashing, salting and rinsing.

A. C. Dahlberg

Also see abs. no. 173, 181, 182.

DAIRY PLANT MANAGEMENT AND ECONOMICS

167. The future of milk consumption. L. SPENCER, Cornell Univ. *Milk Dealer*, 38, 2: 50-62, 86-87. Nov., 1948.

The outlook for milk consumption generally is favorable. The two factors that will have most influence upon future trends of milk consumption are changes in population and fluctuations in consumer incomes. The long-time trend appears to be in the direction of fewer children and more old people, so there is need for more emphasis on the value of milk in the diets of adults, especially those past middle age. During the past few years consumer incomes and purchasing power have been greater than ever before. This is the main reason for the unprecedented rate of milk consumption per capita. The foreign-aid program and large military expenditures are expected to take up any slack in business activity that might otherwise appear during the next year or two. Business forecasts beyond that point are extremely hazardous, but there is good reason to believe that any major depression such as was experienced in the early 1930's would be prevented by government actions of various kinds. Loss of purchasing power by substantial numbers of people whose incomes fail to rise with the cost of living will have a depressing effect upon milk sales if inflation continues.

Other important but minor factors in the outlook for milk consumption are retail prices of milk, the maintenance of satisfactory service and quality, consumer acceptance of milk as a food and the consumer's attitude toward the milk industry. Much more effort should be made to inform consumers about prices, costs and profits in the industry as well as about the food value of milk.

C. J. Babcock

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

168. Carotene retention in alfalfa meal. Effect of blanching, packaging, and storage tempera-

ture. W. L. NELSON, J. K. LOOSLI, G. LOFGREEN AND N. YAGER. *Ind. Eng. Chem.*, 40, 11: 2196-2198. Nov., 1948.

The blanching of alfalfa before dehydration resulted in the retention of appreciably more carotene in the dried meal. Samples so treated contained an average of 164 μ of carotene per g. of dry alfalfa meal as compared with 43 μ for similar samples of unblanched alfalfa. Blanching pretreatments had no influence on the retention of carotene during storage. The use of moisture vapor-proof laminated foil bags was less effective in preserving carotene either at room temperature or at 40° F. than were cloth or paper bags. The addition of calcium oxide to maintain low moisture in the samples appeared detrimental to carotene retention. Alfalfa meals stored at 40° F. in cloth or paper bags showed a marked rise in moisture content during storage periods up to 6 mo.

B. H. Webb

Also see abs. no. 170.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

169. **Experience with pen stables.** F. W. GRAVES, N. Y. Dept. of Health, Albany. *Ann. Rpt. N. Y. State Assoc. Milk Sanit.*, 21: 29-34. 1948.

Most dairymen with pen stables are pleased with them. The cows remain cleaner and have less udder trouble, fewer leg and shoulder injuries, and fewer rheumatic ailments. Cows in heat are observed more easily. There is a saving in labor for cleaning barns and in feeding.

Unfavorable experiences include too little bedding, resulting in unclean animals. There ought to be at least 10 ft. of head room and at least 70 ft.² of floor space for a Jersey and small Guernsey cow, 80-90 ft.² for a medium-sized cow and 100 ft.² for a large Holstein, Brown Swiss or Shorthorn cow. It sometimes is difficult to remove enough posts in remodeled barns to permit the use of tractors and power forks. Cows with horns cannot be stabled safely in pen barns. The pen barns do not display individual cows to advantage.

Today there are 20 pen stable barns in operation in N. Y. State and more have been approved. The quality of the milk has not been affected by pen stabling, but the number of tests is too small for a final conclusion.

A. C. Dahlberg

170. **Calf feeder.** C. I. YOUNG. U. S. Patent 2,455,848. 3 claims. Dec. 7, 1948. *Official Gaz. U. S. Pat. Office*, 617, 1: 246. 1948.

A bottle with a nipple is supported in a cage which is hinged on the wall to facilitate removal.

R. Whitaker

171. **Overhead support for milkers.** H. B. BABSON (assigned to Babson Bros.). U. S. Patent 2,454,300. 11 claims. Nov. 23, 1948. *Official Gaz. U. S. Pat. Office*, 616, 4: 986. 1948.

A stall with an overhead framework designed to support a mechanical milker in position beneath the cow is described.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

172. **Liquid sugar in food products.** W. R. JUNK, O. M. NELSON AND M. H. SHERRILL. Calif. and Hawaiian Sugar Refining Corp., Ltd., San Francisco, Calif. *Food Tech.*, 1, 4: 506-548. Oct., 1947.

Some of the more useful published data in the literature together with some original unpublished material of the authors on invert sugar are presented in this paper. Tables and graphs showing weights per gal., refractive indices, viscosities, boiling points, freezing points and other information about sugar solutions are given. To obtain a minimum of color in the finished syrup, the sugar solution should be inverted at the lowest practical temperature and neutralization of the acid in the inverted syrup should be delayed as long as possible before cooling is completed.

E. R. Garrison

173. **Mix cooling—a problem in small production units.** F. HUMPHRISS, A.R.I.C. *Dairy Ind.*, 8, 10: 1021-1022. Oct., 1948.

The Ice Cream Regulations (England), 1947, regarding heat treatment, etc., require that all ice cream mix be cooled to 45° F. or lower within 1.5 hr. after pasteurization and held there or below until frozen. Suggestions for mechanical cooling are given, especially for the small manufacturer. Documentary evidence that cooling apparatus is in order constitutes a successful defense against accusations that the Heat Treatment Regulations are not being complied with until May, 1949. This reflects the difficulty in England of securing dairy equipment.

G. H. Watrous

174. **Selling ice cream through vending machines.** CLARON BURNETT, Ice Cream Review. *Ice Cream Rev.*, 32, 6: 34, 66. Jan., 1949.

Factories may prove to be one of the most

important outlets for ice cream cups and bars sold through vending machines. In one group of Milwaukee, Wis., plants, ice cream vending machines have increased sales and have resulted in less interruption in work schedules than when ice cream was sold by vendors passing through the plants. At these plants 25 vending machines for 10¢ chocolate-coated bars are in current operation with 50 more machines to be installed this spring. The installation of one machine for each 200 persons is considered profitable. Daily sales per unit have ranged from \$12 to \$20. When the number of units is increased to 75 it is estimated that the total sales will average approximately \$1,500 per day.

Electric current consumption for the ice cream vending machine was lower than for soft drink vending machines, because the ice cream has been hardened before it is placed in the machine. Electric current for the operation of the machines is furnished by the industrial plants. One cent from each ice cream bar sold goes into an employee fund and the remaining receipts are retained by the company which operates and services the vending machines.

W. J. Caulfield

175. Packaged sundae. F. T. MOSHER. U. S. Patent 2,435,094. 1 claim. Jan. 27, 1948. Official Gaz. U. S. Pat. Office, 606, 4: 627. 1948.

Ice cream from the freezer is placed in a small carton, covered with a layer of fruit or other flavored heavy syrup and closed. The packaged sundae then is hardened while inverted.

R. Whitaker

176. Ice cream scoop. J. J. DEUTSCH AND J. VIGILANTE. U. S. Patent 2,454,735. 7 claims. Nov. 23, 1948. Official Gaz. U. S. Pat. Office, 616, 4: 1094.

A wiper, pivotally mounted in a hemispherically-shaped bowl, is made to rotate by rotating a sleeve on the handle of the dipper.

R. Whitaker

Also see abs. no. 164.

MILK

P. H. TRACY, SECTION EDITOR

177. Recent amendments to the U. S. Public Health Service milk code. A. W. FUCHS, U. S. Publ. Health Service, Washington, D. C. Ann. Rpt. N. Y. State Assoc. Milk Sanit., 21: 135-150. 1948.

During the war the 1939 edition of Public Health Bull. 220 remained in effect and a special committee was appointed in 1947 for the purpose of revision. Provisions of the sanitary code

were made enforceable by degrading and by court suspension. Considerable material that was not actually public health was removed from the text and placed in the appendix. Several changes were made in computing sanitary ratings. The code provides for emergency sale of ungraded milk, labelling of Vitamin D milk, and bacterial and temperature standards. Several changes have been made in bacterial standards, including a maximum coliform count of 10 per ml. on pasteurized milk.

All revisions were published in a tentative report in 1947 and the final code will be published in 1948.

A. C. Dahlberg

178. Let's sell more fat-free milk. W. L. FOUST, Warren Sanitary Milk Co., Warren, Ohio. Milk Dealer, 38, 1: 44, 80. Oct., 1948.

A description is given of Vitolac, a low-fat milk prepared by concentrating skim milk under high vacuum and low temperature until the total solids are roughly 12.5%. After standardizing to 1.5% B.F., 2,000 units of vitamin A and 400 units of vitamin D are added. The product tastes richer and slightly sweeter than regular milk. A table is presented which shows the comparative nutritional values of milk and Vitolac.

C. J. Babcock

179. The influence of conditions of cold storage prior to distribution on the keeping quality of pasteurized milk. G. M. PHILLIPS, Univ. College of Wales, Aberystwyth. Proc. Soc. Applied Bact., 1946, 1: 40-42. 1946.

Duplicate samples of milk were taken from the filler, subjected to the 0.5-hr. methylene blue and keeping-quality tests. Then one sample was stored in a laboratory refrigerator at 36-43° F. and the other in a cold storage room in which the temperature rose to 50-60° F. on the afternoon of bottling but was 39-43° F. on the following morning. After 24 hr. storage the same 2 tests again were performed. Those stored in the laboratory refrigerator all were satisfactory by the methylene blue test and only 2.3% had a keeping quality less than 48 hr. Of those stored in the cold storage room, 30% reduced methylene blue in less than 0.5 hr. and 57% had a keeping quality of less than 48 hr. A similar deterioration of quality was observed when the milk was cooled to only 44-50° F. before bottling, although subsequent storage was at 36-43° F.

M. L. Speck

180. Visual conditions on the farm as an indication of the keeping quality of milk. D. A. MCKENZIE AND D. A. BOWIE. Dept. of Agr., Univ. of Leeds. Proc. Soc. Applied Bact., 1946, 1: 35-36. 1946.

A study was made of the bacteria present in

milk and on the equipment of farms having adequate and inadequate facilities for the normal production of high quality milk. The "poor type" farms selected for study consistently produced good milk, and the "good type" farms consistently produced poor milk, as judged by the resazurin reduction tests and keeping quality tests. Milk and swabbings of equipment from the "poor type" farms showed a predominance of micrococci and gram-negative, alkali-producing rods which had little effect on the tests used. Similar samples from the "good type" farms showed a predominance of streptococci and gram-negative, acid-producing rods which have marked influence on the tests used. These differences were believed to be caused by the greater opportunity for contamination offered on the "good type" farms by the more elaborate equipment used.

M. L. Speck

181. Types of farm milk coolers. R. C. SHIPMAN, United Coop. Lab., Ithaca, N. Y. Ann. Rpt. N. Y. State Assoc. Milk Sanit., 21: 165-183. 1948.

This discussion applies to cooling milk in cans. This method is most generally used on small and average-sized dairy farms. Thermocouples were placed at various positions in the milk and in the water in the cooling tank. Cooling was relatively slow in milk placed in unagitated water, especially the top milk in the can if the cooler were only partially filled. Agitation of the water for an hour gave prompt cooling below 50° F., providing the water level was sufficiently high.

A. C. Dahlberg

182. Spray cooling apparatus for milk cans. D. DONNELLY (assigned to Universal Milking Machine Co.). U. S. Patent 2,455,162. 15 claims. Nov. 30, 1948. Official Gaz. U. S. Pat. Office, 616, 5: 1354. 1948.

Freshly-drawn milk in cans is cooled by placing the cans in an insulated tank containing water cooled by submerged expansion coils. A pump circulates the water to a pan which delivers it to the neck portions of the milk cans.

R. Whitaker

Also see abs. no. 148, 149, 150, 152, 153, 154, 161, 167, 182, 187.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

183. Studien über den Mechanismus der Milchbildung. (Studies concerning the mechanism of milk secretion.) English summary. W. FARRZ. Die Milchwissenschaft, 3, 3: 65-75; 3, 4: 97-102. 1948.

After obtaining evidence against the 2-phase

milk secretion theory, the author investigated the 1-phase theory and was able to determine the rate of milk secretion in a lactating animal. Milk cows during their 2nd to 3rd month of lactation showed the highest rate of milk secretion per hr. during the 4th hr. from time of previous milking, whereas cows during their 7th to 10th mo. showed the highest rate during the 7th hr. Assuming the maximum milk-holding capacity of the secretory glands and the maximum pressure exerted upon the glands to be constants, the secretory glands held 42% of the milk obtained in one milking.

The rate of milk secretion per lactation period with 2 or 3 milkings daily when plotted on a graph follows the curve of a quarter ellipse. Immediately after each milking the rate of secretion starts from zero, no matter what the rate was at the onset of milking. Total milk yield per lactation period was highest with 4 milkings daily, as compared with 2, 3 or 5 milkings, provided that the animal is healthy, milk secretion is not disturbed, abundant feed is available and a normal lactation period of 300 d. is practiced. Evidence was obtained that 4 hr. is the minimum filling time of the secretory glands. Milking only twice daily reduces total milk yield per lactation period by 30 and 34% as compared with 3 and 4 daily milkings, respectively. For optimum milk yield per lactation period, 4 milkings daily should be practiced for the first 5 mo., followed by 3 milkings for the next 4.1 mo., and reduced to 2 milkings from then on unless the lactation period is to terminate in 10 mo., in which case only 1 milking daily should be carried out for the last 27 d.

A decrease in feed consumed by an animal results in a directly proportional decrease in milk yield if the cow is milked 3 times daily; at 4 milkings daily the decrease in milk is more pronounced and at 2 milkings less pronounced than at 3 daily milkings, indicating in the last case that substances are withdrawn from the animal body which may weaken the latter and impair its health.

The article presents (a) derivations of formulas for calculating rate of milk secretion at different stages of lactation, (b) milk yield variation with frequency of milking, (c) effect of subnormal feeding and of irregular milking intervals upon milk yield.

I. Peters

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

184. Milk in human nutrition. C. P. SEGARD. Wisconsin Alumni Research Foundation. Milk Dealer, 38, 3: 114-118. Dec., 1948.

The four great discoveries in the field of hu-

man health within the past century are: (a) discovery of anesthetics, (b) discovery of antiseptics, (c) cause and control of epidemics and (d) the vital importance of nutrition in the prevention of disease.

Milk contains the four basic groups of nutritionally important substances, namely carbohydrates, proteins, fats and minerals and vitamins. The most important mineral element needed by the human body is calcium. The growing child needs fully one-fourth of a teaspoonful of calcium daily, and the adult about one-third less. Milk is the only food taken in average serving quantities that can supply this amount. Iodine, copper, iron, manganese, magnesium and others also are needed and are present in milk. Milk sugar or lactose, which accounts for about 30% of the energy value of milk, is in readily assimilable form. As each new vitamin has been discovered, its presence in milk has been shown. The cream, or milk fat, is not only an important energy food but it is the only fat, with the exception of fish oils, that contains four vitamins. Milk may be fortified with vitamins, and the product most recently introduced is fat-free milk fortified with vitamins A and D.

C. J. Babcock

Also see abs. no. 178.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

185. Detergents for dairy plants and methods of their evaluation. H. G. HARDING and H. A. TREBLER. Natl. Dairy Research Labs., Inc., Baltimore, Md. Food Tech., 1, 3: 478-493. July, 1947.

The important requirements of a good dairy cleaner are enumerated and the general characteristics of several washing compounds are discussed. Alkaline detergent mixtures for dairy plants should contain sufficient polyphosphate to prevent precipitation of the calcium, magnesium and iron salts from the water and milk residues, sufficient wetting agent to promote contact between the soil on the equipment and the wash solution and sufficient alkalinity to dissolve denatured protein residue. The most desirable composition of the dairy cleanser will vary with a number of factors, particularly with the type and amount of soil to be removed from the equipment and with the cost of the different chemicals. A method is given for calculating the composition of detergents for specific uses. Graphs showing the influence of hardness (from soil and water) on the composition and cost of recommended cleaning solutions are given.

Tests for pH, alkalinity, surface tension and turbidity-preventing power under simulated plant conditions are recommended to indicate performance quality of detergents.

E. R. Garrison

186. Modified non-ionic synthetic detergents and quaternary ammonium compounds as cleaner sanitizers in food and dairy operations. G. J. HUCKER, N. Y. Agr. Expt. Sta., Geneva. Ann. Rpt. N. Y. State Assoc. Milk Sanit., 21: 35-52. 1948.

Studies for the past few years at Geneva have been focused on the development of a mixture of compounds that would efficiently clean and sterilize in one operation with cold water. Only the non-ionic synthetic detergents with quaternary ammonium compounds offer possibilities of success. Non-ionic detergents without agitation removed about 50-60% of the soil from aluminum or glass; with agitation about 70% was removed, but when modified by the addition of special compounds, 100% of the soil was removed. When the soil was dried on the hard surface, then removal was difficult.

Selected quaternary ammonium compounds were effective sterilizers without odor. They were effective in the cleansing solution against the usual test bacteria, including *Pseudomonas fluorescens* which often has been found so difficult to kill. The unmodified non-ionic detergents and quaternaries were not effective, however. The modified non-ionic detergent-quaternary solution was used as a cleaner-sterilizer for milking machines. "Dairymen were instructed at the premises to draw three gallons of the mixtures through the machine once daily and also once daily to use a brush on all rubber parts." The milking machines were clean and the milk gave low total and thermotolerant bacterial counts.

A. C. Dahlberg

187. Cleaning and sterilizing milk tank trucks. J. R. PERRY, Sealtest Inc., New York. Ann. Rpt. N. Y. State Assoc. Milk Sanit., 21: 95-110. 1948.

The problem of cleaning and sterilizing may be grouped as (a) cleaning and sterilizing facilities and equipment, (b) cleaning and sterilizing procedure, (c) sanitary control, (d) construction of tanks and (e) design of tanks. Details of the cleaning and sterilizing procedures are presented. Sealtest has developed several aids in the cleaning operation. These include water mechanically controlled at 115° F., light, flexible hot water hose, control of the water pressure, an automatic hose water-shut-off valve, and a

special washing solution-fed brush. A portable cleaning solution tank has been developed to maintain the solution at 115° F. and under constant pressure. A special tank with rotary brush has been developed for washing sanitary fittings and pipes. A special milk tank truck has been constructed which should simplify cleaning.

A. C. Dahlberg

188. Restaurant sanitation. GEORGE WEST, Rochester, N. Y., Health Bureau, Rochester, N. Y. Ann. Rpt. N. Y. State Assoc. Milk Sanit., 21: 157-164. 1948.

The significance of food in outbreaks of disease is shown by statistics of the U. S. Publ. Health Service from 1938-1945, inclusive, showing in the United States that there were 12,102 individual cases of illness due to milk, 111,839 due to water, and 57,591 due to food. Over half of the food-borne illness occurred from public eating places. The author discussed the prob-

lem of regulations prescribing proper sanitation and clean food for public eating places.

A. C. Dahlberg

Also see abs. no. 151, 157, 158, 163, 166, 180.

MISCELLANEOUS

189. Symposium on food technology. Ind. Eng. Chem., 40, 12: 2241-2257. Dec., 1948.

Four excellent review papers are presented on different phases of food technology as follows: Sterilization of Foods, J. M. Jackson and H. A. Benjamin, Amer. Can Co., Maywood, Ill., pp. 2241-2246; Processing Edible Fats, Warren H. Goss, Pillsbury Mills, Inc., Minneapolis 2, Minn., pp. 2247-2251; Frozen Food Industry, Clifford F. Evers, Natl. Assoc. of Frozen Food Packers, Wash., D. C., pp. 2251-2253; Measurement of Food Acceptance, E. C. Crocker and L. B. Sjöström, Arthur D. Little, Inc., Cambridge, Mass., and G. B. Tallman, Mass. Inst. of Tech., Cambridge, Mass., pp. 2254-2257.

B. H. Webb

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEWS

190. **Principles of food freezing.** W. A. GORTNER, F. S. ERDMAN and NANCY K. MASTERMAN. John Wiley & Sons, Inc., New York, N. Y. 281 pp. \$3.75. 1948.

Although this book contains comparatively little directly about dairy products, the chapters concerned with the engineering of food freezing contain considerable material on the design and operation of refrigeration facilities which should be of interest to people in the dairy industry because of the straightforward presentation. For those who are operating locker plants in conjunction with dairy enterprises or are marketing frozen foods, considerable information on processing, holding and utilization of miscellaneous frozen foods is presented. The book is not an exhaustive treatise but rather a useful summary of the frozen food industry written in reasonably non-technical language. The format is good and the indexing appears to be better than one sometimes finds in a book of this type. F. E. Nelson

191. **Food plant sanitation.** M. E. PARKER. McGraw-Hill Book Co., Inc., New York, N.Y. 447 pp. \$6.00. 1948

Although written for the entire food industry, this book contains considerable information directly applicable to the dairy industry. All phases of sanitation are covered, from the design of the plant and the sanitary quality of the water supply to the placing of the food in a package which will provide good sanitation during marketing. Detergency and insect and rodent control are treated at some length. The establishment and maintenance of sanitary practices and the proper training of personnel are discussed. An appendix section on "Excerpts from U. S. Laws and Regulations" is included. This book should be of considerable value to those concerned with sanitation problems in the dairy industry. F. E. Nelson

192. **The chemistry and technology of enzymes.** HENRY TAUBER. John Wiley & Sons, Inc., New York, N. Y. 550 pp. \$7.50. 1949.

This volume supersedes *Enzyme chemistry and*

Enzyme Technology by the same author; therefore, the first part is concerned with the chemistry of enzymes and the second part with their technology. This treatment has led to some duplication—almost replication in several instances—while pertinent material has not been included in other cases, probably to keep this book at a reasonable size. A considerable mass of material is presented and citations to the original literature are numerous. Applications to a wide variety of fields are included. The chapter on "Enzymes in Dairy Products" includes a short and relatively inadequate description of the milk phosphatase enzyme and its use for control of pasteurization. No material on milk lipase is presented. Cheese ripening is discussed in 6 pages, probably adequate space for this type of publication if the choice of material had been better. Prevention of mold growth on the surface of Cheddar cheese is ascribed to surface salting. The book contains quite a few errors in the naming of organisms and the same organism is discussed under more than one name in some instances. This book probably is another indication that many fields formerly considered quite specialized are becoming so complex that only rarely will one person be able to encompass with complete adequacy all areas which should be included in a discussion of the entire field. F. E. Nelson

193. **Milk and milk processing.** B. L. HERRINGTON. McGraw-Hill Book Co., Inc., New York, N. Y. 343 pp. \$3.75. 1948.

This book was written as a text for a sophomore course in general dairy manufacturing and thus presupposes a knowledge of general chemistry. As would be expected from the background of the author, the chemical approach is emphasized in presentation of the material. The first part of the book is concerned with the composition of milk and the nature of the different constituents. Chapters on microorganisms, pasteurization, creaming and cream separation, and homogenization are followed by discussions of the various dairy products. Chapters on the Babcock test and lactometry follow. The index seems adequate for a book of this type. The material is pre-

sented logically and in an easily understood form. This book should be very useful not only as a text for a course in general dairy manufacturing but also as a reference book for plant operators with reasonably good technical background.

F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

194. Field studies on bovine venereal trichomoniasis effects on herds and efficacy of certain practices in control. D. E. BARTLETT AND G. DIKMANS. Bureau of Animal Industry, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, 10, 34: 30-39. Jan., 1949.

An analysis of the effect of trichomoniasis upon the breeding efficiency of a herd is made with detailed protocol of 2 affected herds. A graphical presentation of reproductive records in each herd clearly shows the progressive nature of the disease in the herd and the rapid recovery following institution of control measures. Effective control measures are discussed in detail and a graphic outline is given to emphasize the important steps in the hygienic breeding program. The basis of the program is accurate diagnosis of the infectious state of each animal in the herd, use of clean sires and sexual rest for infected females. Eight of nine herds following this program have recovered satisfactorily from trichomoniasis.

E. W. Swanson

195. Further observations on experimental treatments of *Trichomonas foetus* infection in bulls. D. E. BARTLETT. Bureau of Animal Industry, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, 9, 33: 351-359. Oct., 1948.

The intravenous administration of NaI was successful in eliminating *T. foetus* infection in 2 of 8 trials. Other treatments with iodides are reviewed and a seasonal trend in their efficiency is suggested. Intrapreputal insufflation with a silver protein powder and direct application of a trypanflavin ointment were ineffective. Topical application of a German-manufactured product, containing trypanflavin, Surfen A, triethyleneglycol and a condensation product of ethylene oxide and water was effective in 7 of 8 trials. The latter was the only effective topically applied material encountered.

E. W. Swanson

196. A nonantigenic allergic agent for intradermal brucellosis tests. H. E. OTTOSEN AND N. FLUM. State Serum Laboratory, Copenhagen, Denmark. Am. J. Vet. Research, 10, 34: 5-11. Jan., 1949.

A method for the preparation of an extract

from *Brucella abortus* by acid hydrolysis is described in detail. The extract did not promote complement-fixing antibodies following intravenous injection, but it elicited strong allergic reactions in *Brucella*-infected animals. Good correlation was observed between the intradermal test in cattle and the blood agglutination test.

E. W. Swanson

197. Studies on the pathogenicity of *Brucella suis* for cattle I. F. V. WASHKO, L. M. HUTCHINGS AND C. R. DONHAM. Purdue Univ., Lafayette, Ind. Am. J. Vet. Research, 9, 33: 342-350. Oct., 1948.

Of 9 cows fed aborted swine fetuses or exposed to *B. suis*-infected swine, *B. suis* was recovered from only one at parturition. Cows previously exposed to *B. suis* did not show significantly greater resistance to *Brucella abortus* than did unexposed control animals. Attempts to establish *B. suis* infection in the bovine udder were successful following introduction of the organisms into the teat canal by injection or by applying to the teat orifice, but were unsuccessful following application of *B. suis* to abraded udder skin, feeding of infected material or exposing of cows to infected swine in a small pen. Acute mastitis resulted from the introduction of *B. suis* into the mammary gland. Elimination of the infectious organisms in the milk was persistent and intermittent throughout the lactation.

E. W. Swanson

198. The *Brucella abortus* ring test. P. A. BRUHN. Royal Veterinary and Agricultural College, Copenhagen, Denmark. Am. J. Vet. Research, 9, 33: 360-369. Oct., 1948.

A short review of the development of the ring test is presented. A specific *B. abortus* antigen dyed with hematoxylin is mixed with milk. The distribution of color between the cream and skim milk layers constitutes the basis of the test. The interference of the Schern-Gorlich reaction (used to detect heated milk) with the ring test is discussed. False positive tests are considered a combination of the S-G reaction and non-specificity of the antigen. Extensive field use of highly specific antigens showed the ring test to be about 80 to 95% accurate. Greater accuracy was secured with pooled herd milk than with individual-cow samples, due largely to variations in creaming of the latter. The development of a highly specific antigen for the ring test is discussed briefly. This antigen gave positive results on pooled milk diluted 1:128, compared to a sensitivity of only 4-fold dilution with a whey agglutination test. Evidence is presented that consistently positive ring tests indicate infection even in the presence of extremely low blood

titers. The use of the ring test is proposed as a means of more effectively applying a brucellosis eradication program on an area basis.

E. W. Swanson

199. Effect of sulfamethazine on certain infections of the bovine mammary gland. O. W. SCHALM, R. A. BANKOWSKI, R. W. ORMSBEE, AND T. W. BROWNE. Univ. of California, Berkeley. Am. J. Vet. Research, 10, 34: 56-62. Jan., 1949.

Sulfamethazine was given intravenously, followed by 3 daily oral doses to a group of 10 cows infected with *Staphylococcus aureus*, *Aerobacter aerogenes*, and *Streptococcus agalactiae*. No significant change in the udder infection or character of the milk occurred. Levels of sulfamethazine in the milk were considered too low, so a series of 6 semi-daily intramammary infusions were given to 7 infected cows. Infection persisted in all of the cows following treatment. Milk was more abnormal following treatment than before, although production was not decreased. Oral treatment of 8 dry cows was most successful in establishing desired udder concentrations of sulfamethazine, but it was not effective in eliminating *S. aureus* and *A. aerogenes* infections. Treatment of acute and chronic cases of *A. aerogenes* and *Pseudomonas aeruginosa* mastitis with sulfamethazine also was ineffective.

E. W. Swanson

200. Bovine mastitis. Economic importance of the disease—methods of detection, control and prevention. G. R. SPENCER. Univ. of Wisconsin, Madison. Milk Plant Monthly, 37, 12: 35-36, 77. Dec., 1948.

Mastitis, the most costly of all diseases related to the dairy cow, is the most puzzling problem that confronts the dairy industry today. Chronic mastitis which may survive for months and years usually is caused by streptococci, whereas acute mastitis which lasts only a few days commonly is caused by staphylococci and coliform organisms. Although this disease is of no serious threat to public health, the cause of this inflammation must be detected and treated, since health-endangering hemolytic organisms may infect the udder. Proper pasteurization has done much to eliminate this threat.

The microscopic body-cell count and the catalase tests are used widely in the laboratory for detecting mastitis. Herdsmen must rely on physical examination of the milk and animals for detecting the disease. Segregations, sanitation, disinfection and proper milking procedures aid most in controlling mastitis. However, treatment of the animal with penicillin and sulfona-

mides under the direction of a veterinarian has aided in effecting control in certain types of mastitis.

J. A. Meiser, Jr.

201. The results of a mastitis herd survey in the N. E. midland area during 1946-1948. D. L. HUGHES. Vet. Record, 60, 50: 659. Feb., 1949.

In the process of research on penicillin therapy for mastitis, the author examined bacteriologically milk samples from 19 problem mastitis herds involving a total of 591 cows. The types of infection found were as follows: *Staph. pyogenes*, 120 cows or 20.3%; *Str. agalactiae*, 91 cows or 15.2% and *Str. dysgalactiae*, 24 cows or 4.0%. In a few herds a variety of organisms was found such as diphtheroid bacilli, unidentified streptococci and micrococci. The author suggests that the unexpected comparatively low level of *Str. agalactiae* infections in the herds surveyed may be due to the rather widespread use of penicillin therapy in that area or possibly variations because of locality. In a similar investigation undertaken in different counties in 1944-45, a *Str. agalactiae* incidence of 59.3% was found.

R. P. Neidermeier

Also see abs. no. 265, 266.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

202. Packaging of print butter. T. A. NICKERSON AND S. T. COULTER, Univ. of Minnesota. Natl. Butter Cheese J., 40, 1: 26-27, 52, 54-57. Jan., 1949.

This study was made to obtain information on the protection furnished print butter by various combinations of wrapping material against such factors as absorption of odors, oxidation, shrinkage, physical damage and deterioration in the consumer's refrigerator. The heat-sealed pliofilm and cellophane gave the best protection against absorption of odors. However, no one wrapper could protect the butter from all foreign odors. The wrapping material that protected butter from light provided the best security against deterioration caused by oxidation. The degree of oxidation of the butter was determined by flavor scores, as well as by the aldehyde and peroxide values of the fat.

Four 0.25-lb. prints of butter suffered a greater loss due to shrinkage than a 1-lb. print under similar conditions. Foils and pliofilm both gave satisfactory protection against shrinkage as did parchment + plioform + carton. The greatest resistance against physical damage to the butter was afforded by the cardboard cartons. Wrapping materials such as cellophane, pliofilm or parchment gave little protection against external phy-

sical force. Under home storage conditions foil and parchment + carton gave about equal protection to the butter. H. E. Calbert

203. Let's sell butter! ROY ROBICHAUX. Western-United Dairy Co., Chicago, Ill. Milk Plant Monthly, 38, 1: 70-72. Jan., 1949.

A five-point program for promoting the sale of butter is outlined as follows: (1) Use cream of sufficient quality to produce at least a 90 score butter. (2) Use proper processing methods and technics so that the best possible butter is produced. (3) Use appealing efficient packaging methods that will insure the product against outside contamination. (4) Police the consumer outlets to insure that the product is fresh and wholesome when it reaches the consumer. (5) Use only butter of 91 score or better when storing for future consumer use. J. A. Meiser, Jr.

204. De invloed van het vetgehalte van den room op de stevigheid, het vochtgehalte en andere eigenschappen van de boter. (The influence of the fat content of the cream upon the consistency, the water content and other properties of the butter.) (English summary.) H. MULDER. Rykslandbouwraproefstation, Hoorn, Holland. Landbouwkund. Onderzoekingen, 52, 6C: 268-276. 1946.

Butters were made from creams with different fat content under the same temperature conditions and other conditions as much the same as possible. Fat contents of 25-50% were compared with 8-12%. The thin cream took a much longer churning time, butter granules grew very slowly and needed a long working time. These butters contained more moisture and buttermilk than the other butters. There was no difference in hardness.

The fat content of the buttermilk was about the same, causing high fat losses in the cases of thin cream. The phospholipid content of the buttermilk was about proportional to the fat content of the cream. More fat could be separated out of the buttermilk from the thin cream. This cannot be explained by the different phospholipid content. After separating the buttermilk, the fat loss with thin cream still remained higher.

A. F. Tamsma

205. Onderzoek naar de mogelykheid van verbetering van de stevigheid van zomerboter. (Investigation into the possibility of increasing the firmness of summer butter.) W. ADRIANI AND A. F. TAMSMA. Laboratorium der Coop. Fabriek van Melkproducten, Bedum, Holland. Landbouwkund. Onderzoekingen, 47, 12G: 941-1024. 1941.

Experiments were made to ascertain how the

firmness of butter (measured at 18° C.) is influenced by the manufacturing process. Souring at 18° C. had no advantage against souring at normal temperature (14° C.); sometimes it caused the fat content of the buttermilk and the water content of the butter to be too high. Both too cold and too warm wash-water led to softer butter. Souring, churning and washing at about 14° C. gave the best results.

To get more insight, the crystallizing of butterfat was studied by the dilatometric method. During cooling, a very pronounced undercooling phenomenon takes place. By cooling butterfat gradually and afterwards increasing the temperature little by little, in the diagram representing the quantity of solid phase as dependent upon the temperature, a loop is described so that the quantity of solid phase at a given temperature is higher when this temperature is reached from a lower temperature rather than from a higher temperature. Cooling in steps leads to a smaller quantity of solid phase. At a given temperature, for instance 18° C., one gets the largest quantity of solid phase by cooling melted butterfat immediately to a lower temperature, e.g., 13 to 14° C., and raising the temperature to 18° C. only after the fat has remained a sufficiently long time at the lower temperature.

The theory was set up that firmness of the butter depends in the first place on the quantity of solid phase (crystallized butterfat) and in the second place on the structure of this solid phase. From this theory it follows why souring, churning and washing at temperatures above 14° C. had an adverse effect on the firmness of butter (measured at 18° C.) and why cold wash water led to softer butter. The results of the present investigation could be explained in this way. Also, the results of some experiments which are described in the literature, but which could not be explained in another way, could be interpreted easily by this theory and the fundamental facts mentioned previously.

One must prevent the butter from reaching (even temporarily) a higher temperature. Too high temperatures proved very disadvantageous and cooling to a lower temperature after exposure to high temperatures did not restore the original firmness.

The temperature of the butter is of great importance for the firmness, because the temperature coefficient is large, a difference of 10% in firmness corresponding to a temperature difference of only 0.25° C. A. F. Tamsma

206. Onderzoek naar de mogelykheid van verbetering der consistentie van winterboter. (Investigation into the possibility of improving the texture of winter butter.) (English and German summary.) W. ADRIANI AND A. F. TAMSMA.

Laboratorium der Cooperatieve Fabriek van Melkproducten, Bedum, Holland. Landbouwkund. Onderzoekingen, 52, 1G: 1-24. 1946.

A good texture of winter butter may be obtained in choosing the right manufacturing process, taking care that the structure leading to brittleness is destroyed and that the percentage of crystallized fat in the butter at a given temperature, e.g., 13° C., is as low as possible, as a high percentage of crystallized fat goes together with a high hardness of the butter.

The first point is effected by working at low temperature, which can be reached by washing the butter three times with washing water of 9° C. or a little lower still. The second point is effected by cooling the cream in successive steps. In a laboratory investigation keeping butterfat at different temperatures and determining the percentage of crystallized fat by the dilatometric method, it was established which temperature steps were the most effective for a good texture of the butter at 13° C. The results obtained were confirmed by experiments on a technical scale in the factory, proving the following manufacturing process satisfactory. The pasteurized cream is cooled at 14° C. After some hours the temperature is raised to 22° C. and maintained during an hour. Then the cream is cooled to 14° C. and soured at this temperature overnight. The ripened cream is churned at 14° C. and the butter washed and worked as described above.

A. F. Tamsma

207. *Verschillen in stevigheid by Zomerboters door verschillende fabrieken bereid.* (Differences in firmness of summer butters, produced by different factories.) (English, French and German summaries.) W. ADRIANI AND A. F. TAMSMA. Laboratorium der Cooperatieve Fabriek van Melkproducten, Bedum Holland. Vakgroep Boterindustrie. The Hague, Holland. 23 pp. 1946.

Determination of the firmness of summer butter at 18° C., rather than at 13° C., is advocated for two reasons: (a) The firmness of summer butter is of most interest at the higher temperature. (b) At 13° C. the systematic differences between the factories were partly and sometimes completely covered by the great fluctuations that, on the strength of theoretical considerations derived from study of butterfat, were expected to occur in a much smaller degree at higher temperature. Statistical examination of all data available at different factories showed that if differences in fat composition were taken into account, there were still some factories producing butter which on an average was firmer, other which was softer than that corresponding with

the calculated line of regression. These differences could be explained sufficiently by differences in method of manufacture according to the facts established by the investigations of the authors. The composition of the milk fat affected the firmness of the summer butter of different factories much more than did the method of manufacture.

A. F. Tamsma

CHEESE

A. C. DAHLBERG, SECTION EDITOR

208. *Smaak en smaakstoffen van kaas.* (Taste and taste compounds of cheese). H. MULDER. Landbouwkund. Tijdschr. 59, 706/708: 181-187. March/May, 1947.

After an introduction on the history of different kinds of cheese, the author comes to a description of taste, particularly taste of cheese. The different components of cheese, as far as they contribute to the taste, are discussed. The equilibrium and cooperation of the taste components are considered important. Nomads probably made the first cheese for army supply. Afterwards educated peoples developed the taste. Later on the existence of different kinds of cheese was governed more by economic than by taste reasons.

The simple differentiation of taste into sweet, sour, salt and bitter is found not adequate, taste being much more complicated. The taste of cheese has a general component but is very variable, because of the many other components. Fat, protein, milk sugar, with their degradation products, milk salts, salt and water all contribute to the taste. Fat is very important as by hydrolysis fatty acids are formed, causing probably a main part of the cheese flavor and taste. Proteins in the ripening process form many compounds contributing to the taste of cheese. Particulars were described. Milk sugar in Dutch cheeses is completely changed to lactic acid, giving a fresh taste, particularly if pasteurized milk was used. Bacteria can form many other products out of lactic acid; the calcium salt of propionic acid causing the sweet taste of Emmentaler cheese comes from this source. All compounds together form the typical cheese tastes. Knowledge of the taste components and the way they are formed is considered very important for the cheese industry.

A. F. Tamsma

209. *Cheese pusher.* F. B. BRANCHFIELD, SR., U. S. Patent 2,457,441. 5 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, 617, 4: 1130. 1948.

An implement for pushing cheese in a cheese vat, consisting of an angular frame and handle

and a screen which may be adjusted to an upright position, is described. R. Whitaker

210. Apparatus for treating cheese. O. F. HANSEL. U. S. Patent 2,455,579. 3 claims. Dec. 7, 1948. Official Gaz. U. S. Pat. Office, 617, 1: 180. 1948.

Cheese of the Blue or Roquefort type is improved by admitting air to the interior through holes punched uniformly in the cheese by a group of small rods which are forced down through the cheese as it is held firmly between 2 plates so perforated to allow passage of the rods. The rods are lowered and raised automatically by an eccentric operated by a source of power. R. Whitaker

Also see abs. no. 214.

CONDENSED AND DRIED MILK; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

211. Gärungsgetränke aus Pflanzenextrakten und Molke. (Fermented beverages from plant extracts and whey). English summary. M. E. SCHULZ AND K. FACKELMEIER. Die Milchwissenschaft, 3, 6: 165-174. June, 1948.

Milone is a sweet-sour, slightly alcoholic fermented drink prepared from plant extracts and whey. Whey is fermented to 1% lactic acid and mixed while cold with an equal volume of 3% herb tea. The tannin in the tea is precipitated by the albumin in the whey and the precipitate removed. The liquid is fermented with lactose-fermenting yeasts and sweetened. The maximum alcohol content of the beverage is 0.8%. The herb tea is made up of leaves of linden, hazel nut, billberry and milfoil, together with spicy herbs such as elder blossoms, rosemary linden blossoms and mint. The technical problems involved in the preparation of milone are discussed in detail and illustrations of a processing plant are given. I. Peters

212. Eighty per cent butterfat cream, its production and use. R. J. SPIERS. Abbotts Dairies, Philadelphia, Pa. Ice Cream Trade J., 45, 1: 44. Jan., 1949.

See abs. no. 87, page A19.

W. H. Martin

213. Treatment of rennet casein for glue manufacturers. A. J. LAWRENCE. Australian J. Dairy Technol., 3, 3: 96-97. July-Sept., 1948.

Rennet casein showing certain quality defects is unsuited for the manufacture of plastics. It also is unsuited for use in glue-making. In a search

for an additional outlet for such casein, it was found that when rennet casein was treated for 1 hr. with 3 parts by weight of 1% sulfuric acid followed by washing 3 times, filtering and drying at 130° F., a casein showing minimum swelling, good color and an ash content of 2.8% was obtained. Glue prepared from such a product met the requirements of 1,000 lb./in.² breaking strain as prescribed in the "Australian Standard Specification for Joiners' Glue (casein) (1941)". Casein treated similarly with hydrochloric acid and subsequently used in the preparation of glue was not satisfactory. J. C. Olson, Jr.

214. Untersuchungen zur Bewertung der Molke. (The determination of the quality of whey.) English summary. G. DEMMLER AND K. KUMETAT. Die Milchwissenschaft, 3, 5: 138-142. May, 1948.

In order to determine watering and/or biological deterioration of whey, various methods were used. The ripening of sweet rennet whey by natural fermentation or by addition of pure homofermenting lactic cultures resulted in a lowering of specific gravity, % of total solids and % lactose, thus making these methods unsuitable for the determination of watering of whey. Whey, which is defined by law as "the liquid obtained in cheesemaking after separation of casein and fat by curdling of milk", should contain not less than 5.5% dry matter when made with acid and rennet, and not less than 5.2% when made with acid alone. Thus whey which is in the normal range of acidity but contains less than the prescribed % dry matter should be regarded as inferior and treated accordingly. Proper cooling and/or pasteurization should be practiced in order to maintain the quality of whey. I. Peters

215. Geschichtliches über Molke und Milchsücker. (Historical data in whey and milk sugar.) English summary. E. FUNCK. Die Milchwissenschaft, 3, 6: 152-160. June 1948.

Documents of the Middle Ages divide milk into butter, cheese and whey. The whey was valued for its nutritional and curative properties. Special attention was given to milk sugar, which was discovered scientifically by Bartoletti in 1633. The name "milk sugar" was used first by Ludavici Testi who used it as a patent name for his medicine against arthritis. In 1711 Fick named the carbohydrate found in milk "milk sugar." Proof that milk sugar is an animal type of sugar was obtained by Scheele in 1780. I. Peters

216. Deformation der Fettkugelnchen in Hochkonzentriertem Rahm mit 80 Prozent Fett. (De-

formation of fat globules in highly concentrated cream with 80 per cent fat.) English summary. W. MOHR AND J. WELLM. *Die Milchwissenschaft*, 3, 6: 149-152. June, 1948.

Microscopic observations of cream with over 75% fat verified the calculated deformation of fat globules at such high concentrations. The deformation of globules means an increase in potential (surface?) energy and, in turn, lowers the stability of the emulsion. In order to escape the pressure causing deformation, the globules can fuse. The fusing is governed by other factors which are responsible for the stability of the emulsion. The deformation of fat globules in 80% cream may denote the first step in butter-making by the Alfa process. I. Peters

217. **Milk turning machine.** J. W. GIBLER. U. S. Patent 2,456,630. 5 claims. Dec. 21, 1948. Official Gaz. U. S. Pat. Office, 617, 3: 763. 1948.

Evaporated milk in cartons stacked on pallets may be inverted easily by one man with this device. Turning is desirable to eliminate cream separation in the stored product. R. Whitaker

218. **Some observations on the bacteriology of condensed milk.** H. B. HAWLEY. Henry Edwards and Sons, Ltd., Pipe-Gate, Near Market Drayton. *Proc. Soc. Applied Bact.*, 1946, 1: 1-5. 1947.

The bacterial count of sweetened condensed milk declined in the course of each day's operation as well as during each weekly period of operation. The high counts occurred immediately after the daily cleaning of the pans and canning machine and after the weekly cleaning of the condensed milk storage vat and pump. These increased counts were considered caused by incomplete cleaning and sterilization of the vacuum pans, packing gland of the pump and the bottom bearing of the agitator in storage vat. The lowering of the count following such periods was caused by the high concentration of solids in the sweetened condensed milk. It was concluded that more attention was needed to constructional design in such plants so that all equipment could be dismantled, cleaned, sterilized and dried.

M. L. Speck

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

219. **Thermophilic and thermophilic bacteria in washed milk churns.** S. B. THOMAS, L. B. JONES, M. A. BEAVAN, AND B. F. THOMAS. Univ. College of Wales, Aberystwyth. *Proc. Soc. Applied Bact.*, 1946, 1: 54-55. 1946.

Milk can rinses showed higher counts at 30

than at 37° C., both before and after laboratory pasteurization at 63° C. for 30 min. The can usually had a high thermophilic count. The 627 thermophilic bacteria isolated from yeastrel milk agar plates incubated at 30° C. were classified as follows: microbacetria, 62.0%; aerobic spore-formers, 18.8%; micrococci and sarcina, 7.5%; gram-negative rods, 7.3%; streptococci, 2.2%; yeasts, 1.4%; actinomycetes, 0.8%. Thermophiles were not encountered frequently in the cans, 3.5% showing counts greater than 500,000/ml., while 77% had counts less than 500.

M. L. Speck

220. **The reproducibility of dye reduction tests with pasteurized milk.** D. W. WATSON, C. J. BESSELL, E. GOLDSCHMIDT, AND J. G. DAVIS. Express Dairy Co., Ltd., 133 Euston Rd., N. W. 1. *Proc. Soc. Applied Bact.*, 1946, 1: 56-58. 1948.

Resazurin gave somewhat better reproducibility in reduction time than methylene blue on replicate samples of raw and pasteurized milk. Both dyes showed better reproducibility with raw than with pasteurized milk. Also the variation in replicate tubes decreased in samples having shorter reduction times. The samples were incubated until complete reduction of the dye occurred, and resazurin was reduced more slowly than was methylene blue in both raw and pasteurized milk.

M. L. Speck

221. **Das Verhalten der Paratyphus-Enteritis-Gruppe in der Molke.** (The behavior of the paratyphoid and enteritis groups in whey.) English summary. R. HEIM AND W. WEDEMANN. *Die Milchwissenschaft*, 3, 5: 133-138. May, 1948.

Samples of rennet and of acid whey were inoculated with pure cultures of the paratyphoid-enteritis group and incubated at room temperature and at 37.5° C. Daily plating on regular agar medium and on Drigalski's medium were made, and the typical colonies obtained examined for motility, gram reaction and agglutination test by appropriate methods. Results of 4 trials with rennet whey showed survival of pathogens up to the 23rd d. in samples held at room temperature, but only up to the 15th d. in those held at 37.5° C., whereas in 7 trials with sour whey no pathogens were isolated after incubation for 7 d. at room temperature and after incubation for 2 d. at 37.5° C. The detrimental effect of acid at higher temperature upon the pathogens is evident. From the health standpoint whey should be pasteurized prior to consumption by humans or animals.

I. Peters

222. **The critical humidity in the destruction of *Streptococcus salivarius* by hypochlorite aro-**

sols. J. WOLF AND A. B. BATCHELOW. Milton Antiseptic and Deosan, Ltd., John Milton House, Brewery Rd., London, N. 7. Proc. Soc. Applied Bact., 1946, 1: 59-61. 1946.

At a temperature of 20-25° C., complete destruction of *Streptococcus salivarius* was obtained in 3 min. at a relative humidity of 56% when 1% hypochlorite aerosol was sprayed to give a concentration of hypochlorous acid gas of 0.5 p.p.m. in the atmosphere. With 45% relative humidity at this temperature, 75% of the bacteria remained. Below 20° C., higher relative humidity was required to obtain an equal kill, while above 25° C. less humidity was necessary.

M. L. Speck

223. An improved medium for the enumeration of bacteria in milk and milk products. M. J. PELCZAR, JR., Univ. of Maryland, College Park, and H. D. VERA, Baltimore Biological Laboratory, Baltimore. Milk Plant Monthly, 38, 1: 30-33. Jan., 1949.

Since the use of the present standard tryptone-glucose-beef extract agar containing milk as a medium for the enumeration of bacteria in milk products offers several disadvantages, the authors propounded a new medium that does not contain meat extract or added skim milk. The composition is as follows: pancreatic digest of casein (Trypticase), 15 g./l.; papaic digest of soy meal (Phytone), 5 g./l.; dextrose, 5 g./l.; sodium chloride, 4 g./l.; cystine, 0.2 g./l.; sodium sulfite, 0.2 g./l.; agar, 15 g./l.; pH, 7.2.

Using standard plating procedures, a series of counts was made on various dairy products. The new medium frequently produced larger colonies than did the standard tryptone-glucose-beef extract agar, yet all counts were comparable. Besides being easily prepared, the new medium, due to its clarity, did not obscure small colonies.

J. A. Meiser, Jr.

224. A method of collecting sterile blood from cattle. F. V. WASHKO. Purdue Univ., Lafayette, Ind. Am. J. Vet. Research, 9, 33: 359. Oct., 1948.

A method is described for obtaining blood from the jugular vein of cattle without contamination. The vein is punctured with a sterile 18-gauge needle passed through a 14-gauge needle used to pass through a disinfected skin area.

E. W. Swanson

Also see abs. no. 198, 218.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

225. Method of treating lactalbumin. G. P. BUTTERFIELD AND L. A. THORNE. (Assigned to

American Seal-Kap Corp.) U. S. Patent 2, 457, 642. 2 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, 617, 4: 1178. 1948.

Dehydrated lactalbumin may be suspended easily in colloidal form in water when made as follows: adjust pH to 7.3 to 8.0, add at 100° F. proteolytic enzymes derived from papain and pancreatic sources, increase temperature to inactivate the enzymes in 1 to 20 min., depending on the strength of the enzymes, and dry to form a stable powder.

R. Whitaker

226. De voorgestelde normalisatie der methoden ter bepaling van de Zuurheidsgraad van melk. (The standardization of the method for the determination of the acidity of milk) English summary. C. I. KRUISHEER. Rykszuivelstation, Leiden, Holland. Netherlands Milk Dairy J., 2, 3: 127-136. July-Sept., 1948.

The principal methods of determining milk acidity (Soxhlet-Henkel, Dornic, Thörner, Mann and Van Norman) use different quantities of milk, sometimes diluted with water. Other differences are the quantity of indicator, the concentration of alkali and the way of expressing results.

Variation in alkali concentration and dilution of the milk can be considered the same factor. A slight amount of water makes little difference (Soxhlet-Henkel, Dornic, Mann), large amounts cause too low and unpredictable values (Thörner, Van Norman) by hydrolysis of calcium caseinate.

Variation in the quantity of indicator causes large differences, especially if small amounts are used. It is best to use a rather large quantity, allowing accurate measurement and giving an easy color change with easily observable end point.

The best way of expressing results would be in decimal units; the number of 0.1 cm.³ of 0.1 N alkali for 10 cm.³ of milk was proposed. By using 0.4 cm.³ of 2% phenolphthalein solution and titrating without dilution, the most desirable form of acidity determination suitable for standardization was obtained.

A. F. Tamsma

227. Variations in milk and their effects on processing. Part I. How variations in the fat and total-solids affect the properties of milk. W. I. TRETSVEN. New Mexico A. and M. College, State College. Milk Plant Monthly, 37, 11: 33. Nov., 1948.

The physical and chemical properties of milk are altered considerably by variation in milk composition. Since the total solids content of milk is relatively small in relation to the moisture content, any change in composition would exert the most pronounced effect on the fat, pro-

tein, sugar and mineral portion of the milk. Milk fat, which is comprised of many fatty acids, shows pronounced variations when analyzed chemically. This fact accounts for fats with high or low melting points, and this in turn alters many plant procedures, such as churning time and temperature, homogenization and separation. Variations in protein content of milk, coupled with the hydrophilic nature of this constituent, exert a pronounced influence on the physical and chemical properties of milk; processing may be altered to a considerable degree.

J. A. Meiser, Jr.

228. Variations in milk and their effects on processing. Part 2. How variations in the proteins, salts, and the other components affect the properties of milk. W. I. TRETSVEN. New Mexico A. and M. College, State College. Milk Plant Monthly, 37, 12: 37, 73. Dec., 1948.

Variations in the protein constituents of milk are not uncommon. Colloidal casein affects the color, viscosity, density and stability of milk, whereas the extremely hydrophilic albumin and globulin influence the creaming properties of milk, especially when exposed to pasteurization temperatures. Variations in lactose composition are not uncommon in mastitis. Also, this component, due to its ease of crystallization, governs ice cream composition to a great degree. Although little is known about the true variations in the salt composition of milk, these changes definitely influence the heat stability and coagulating properties of milk. Increased knowledge of milk components will affect future methods and processing procedures in the dairy industry.

J. A. Meiser, Jr.

Also see abs. no. 213.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

229. Pasteurizing system. R. E. OLSON. (Assigned to Taylor Instrument Co.) U. S. Patent 2,455,605. 3 claims. Dec. 7, 1948. Official Gaz. U. S. Pat. Office, 617, 1: 187. 1948.

The novel feature of this HTST pasteurizing system is a T-shaped fitting having its opposite ends sealed with flexible diaphragms and connected, one with unpasteurized milk as it flows through the heater section and the other with the pasteurized milk as it flows through the cooler. Should the pressure on the pasteurized milk fall below that of the unpasteurized milk, the diaphragms are depressed in such a manner to operate an electrical switch which stops the unpasteurized milk pump, thus preventing any possible leakage of raw to pasteurized milk.

R. Whitaker

230. Method of sterilizing and preserving. A. BRASCH. (Assigned to Electronized Chemicals Corp.) U. S. Patent 2,456,909. 17 claims. Dec. 21, 1948. Official Gaz. U. S. Pat. Office, 617, 3: 833. 1948.

Foodstuffs, including dairy products, may be sterilized without affecting flavor and physical properties, by bombardment with high speed electrons of a velocity equivalent to at least 1 million volts for 0.0001 sec.

R. Whitaker

231. Manufacture of ice cream. Refrigerating Engineering Application Data 41. CHESTER J. BELL, Chairman ASRE Technical Committee on Dairies and Allied Industries. Refrig. Eng., 55: 1. Jan., 1948.

A basic presentation of the varied items of ice cream manufacture covering make-up of ice cream mix, processing ice cream mix, freezing and hardening ice cream is given. Included is a representative flow diagram of ice cream plant equipment employing the continuous freezer, along with operating features and refrigeration applications. Detailed calculations of refrigeration requirements are presented to illustrate refrigeration loads involved in mix cooling, mix freezing and ice cream hardening.

L. M. Dorsey

232. Measurement of holding time of high-temperature, short-time pasteurizers. W. JORDAN AND R. F. HOLLAND. Cornell Univ., Ithaca, N. Y. Milk Plant Monthly, 37, 11: 85. Nov., 1948

Since the margin of safety is exceedingly small in the high-temperature, short-time pasteurizers, accurate timing of the holding period is essential. Injecting varying concentrations of salt (NaCl) into the holding tube and measuring by electrical conductivity the length of time necessary for the salt to be discharged at the far end of the tube showed a saturated solution of salt to be the most satisfactory for determining the holding period. It also was observed that the injected salt solution should be directed across the flow since comparisons of "against-the-flow" to "with-the-flow" produced 0.7 sec. differences in the holding period as measured by this method. Spray-type nozzles were best for injecting the saturated salt solution.

J. A. Meiser, Jr.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

233. Important factors in your operating costs. L. C. THOMSEN. Univ. of Wisconsin. Natl. Butter Cheese J., 40, 1: 74-76. Jan., 1949.

Dairy plant operators must watch operating costs, particularly during period of general de-

pression or inflation. With few exceptions, the dairy industry returns a greater portion of the consumer's dollar to the primary producer than any other industry. To maintain this favorable position great interest is shown in the importance of processing costs.

In a simple method of expressing operating costs, these are classified broadly into the following groups: personal services; material costs; services; general expense; property expense; profit before dividends and interest; taxes. In the dairy industry wages paid to labor have increased approximately 81% since 1939 with little increase in output per individual, indicating a similar increase in labor costs per unit output. A greater output per worker is advocated to compensate for this rise. The minimum figure for profit for the dairy industry should be 2.1% of gross income or 12% of net worth. The ratio of net worth to gross income serves as a means of evaluation of a dairy business. This ratio should be approximately 1:5.7. The plant operator should departmentalize his cost figures to gain the utmost advantage from them.

H. E. Calbert

234. Good will, the hidden treasure in business. FRED MERISH. *Milk Plant Monthly*, 37, 12: 60-62. Dec., 1948.

A discussion of factors necessary to purchase, compute and maintain good will is presented. Two methods for estimating the good will value of a dairy enterprise are presented together with illustrations of the application of the methods.

J. A. Meiser, Jr.

235. Problems in the operation of city milk plants. L. W. HOVR. *Detroit Creamery Co.*, Detroit, Mich. *Milk Plant Monthly*, 37, 12: 104-106. Dec., 1948.

The task of directing and coordinating milk processing today involves nothing more than utilizing sound fundamental plant-operating procedures. This includes such basic items as: (a) regulated receiving, (b) proper production accounting, (c) adequate inventories, (d) utility checks, (e) product waste control, (f) minimized bottle breakage, (g) good housekeeping, (h) product control and (i) proper plant maintenance.

The future necessitates solving additional problems such as: (a) conversion to new type equipment, (b) increasing speed of plant operations, (c) designing more economical methods of warehousing and (d) regulating the quality and appearance of the product to meet new standards.

J. A. Meiser, Jr.

236. Medosweet emphasizes dealers. E. R. LUCAS. *Ice Cream Trade J.*, 44, 12: 44, 45. Dec., 1948.

This Tacoma, Washington, company attributes the steady and consistent growth in consumer acceptance of its product to quality of product, dealer education and point of sale promotion. The dealer education program consists of showing him how to dip ice cream correctly, how to make a profit by correct dipping and suggestive selling and the possibilities of the ice cream department in necessary sales in other departments.

W. H. Martin

237. What consumers think. ANONYMOUS. *Ice Cream Trade J.*, 44, 12: 41, 79-81. Dec., 1948.

The General Ice Cream Corp. of Schenectady, N. Y., assisted by 85 drug store owners in Connecticut, has made a marketing research study involving 9,290 consumers. For a big majority, opening and closing hours were satisfactory and most of the customers said the packaged ice cream was kept hard enough to reach home in a satisfactory condition. Many (31.5%) said that their home refrigerator would not keep ice cream satisfactorily. Fountain service was good, according to 84.5% of the replies and only 2.8% thought it was poor. A majority (54.1%) considered 20¢ a fair price for a good soda and 55.1% thought 25¢ was a fair price for a good sundae. On the question of size of serving, 60.8% indicated a preference for a larger portion priced at 30¢ to a smaller one at 20¢. The information obtained was supplied to dealers with comments on how to increase sales.

W. H. Martin

238. Safeguard your markets. E. J. MATHER. *National Dairy Products Corp.* *Ice Cream Trade J.*, 44, 12: 50, 52, 63, 65, 88-92. Dec., 1948.

The author recommends that manufacturers teach their dealers to price for profit dollars instead of for profit percentages as a means of maintaining and increasing per capita consumption of ice cream.

W. H. Martin

239. Collection time is selling time. R. W. ENGLE. *Engle Dairy*, Philadelphia, Pa. *Milk Plant Monthly*, 38, 1: 34-35. Jan., 1949.

A comparison of every-other-week collection with weekly collection is made. The author maintains that weekly collection allows routemen to contact working housewives more often, thus promoting future sales through personal contact. Every-other-week collection, he maintains, builds up sales resistance since mounting milk bills tend to force the customer to eliminate the extras.

J. A. Meiser, Jr.

240. A customer solicitation program. ROSS MILLER. *Milk Plant Monthly*, 38, 1: 46-47. Jan., 1949.

This program requires that routemen submit a weekly list of 12 prospects or potential customers to the plant office. A 12-page booklet describing the operations of the company plus a form letter is sent to the prospective customer followed by a free bottle of milk. The driver then contacts the recipient 2 or 3 days later. This practice of solicitation has been very successful in adding new customers.

J. A. Meiser, Jr.

241. A year-round incentive plan. TED KNIGHT. Milk Plant Monthly, 38, 1: 60, 65. Jan., 1949.

For each new customer picked up and retained for a 30-day period, the routeman receives a bonus of 50 cents per qt. for the month's average daily delivery to the new customer plus the usual dairy products percentage. Wholesale routemen receive \$2.40 for each case of milk ordered by a new customer for a 30-day period.

J. A. Meiser, Jr.

242. Don't live off your reserves. H. J. ASHE. Milk Plant Monthly, 38, 1: 48, 50. Jan., 1949.

To maintain equipment and rolling stock it is extremely important that a depreciation reserve be maintained religiously; otherwise, earnings will be reduced because of antiquated equipment or accumulated replacement costs.

J. A. Meiser, Jr.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

243. Fermentation ability of ingesta from normal and atonic bovine rumens. E. C. STONE. State College of Washington, Pullman. Am. J. Vet. Research, 10, 34: 26-29. Jan., 1949.

Rumen ingesta from normal rumen-fistulated cows produced from 112 to 250 ml. gas when incubated for 4 hr., compared to 4.5 to 59 ml. from cows with atonic rumens operated on for removal of foreign bodies. Experimental traumatic reticulitis or fasting 48 hr. produced the typical atonic rumen reaction. Volatile acidity of normal rumen ingesta was 84 to 167 ml. 0.1 N, while from atonic rumen ingesta it was 24 to 88 ml. 0.1 N. Volatile acidity varied with time of feeding.

E. W. Swanson

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

244. A summary, breeding failure survey in Washington. R. E. ERB AND A. O. SHAW. State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 40. 1948.

Analysis of 15 dairy herds which had exhibited

a high incidence of breeding difficulties revealed that the following were associated with low breeding efficiency of the cows studied: poor nutrition, disease of the reproductive tract, short interval between parturition and insemination, irregularity of intervals between inseminations and low breeding efficiency of the sire. There was an indication that breeding difficulties were more frequent among cows born in the spring of the year than among those born at other seasons. No significant difference was found in the rate of conception following artificial insemination as compared to natural breeding.

N. L. Jacobson

245. Results of the proved sire program in the Holstein herd at the University of Idaho. D. L. FOUNT AND R. H. ROSS. Idaho Agr. Expt. Sta., Moscow. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 58. 1948.

The continuous selection of sires on the basis of the type and production of their daughters resulted in an increase in the average butterfat production and an improvement in the type of the Holstein herd at the University of Idaho.

N. L. Jacobson

246. Factors influencing the methods of bovine semen evaluation. B. A. ASH AND R. E. ERB. State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 76. 1948.

Studies of undiluted bovine semen revealed that the survival time of sperm at 45° C. was greatest at concentration levels of 800,000 to 1,000,000 sperm per cubic mm. The survival of undiluted semen in storage at 40° F. was correlated positively with survival time at 45° C. and with the ascorbic acid levels of whole semen. A significant positive correlation was found between methylene blue reduction time and ascorbic acid values of semen.

N. L. Jacobson

Also see abs. no. 195.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

247. Milk pail cover. A. C. WEBBY. (Assigned to Solar Corp.) U. S. Patent 2,460,049. 8 claims. Jan. 25, 1949. Official Gaz. U. S. Pat. Office, 618, 4: 1129. 1949.

A milk pail cover having a pair of tubes for attaching the teat cup hoses and a pair for attaching to suction and pulsator is described. One control handle closes the latter and opens the former to permit removal of the cover from the pail for emptying. Turning the handle in the opposite direction reverses the valves and places the equipment in operation.

R. Whitaker

248. Pressure Releaser Milker. F. G. HODSDON. (Assigned to International Harvester Co.) U. S. Patent 2,458,779. 7 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, 618, 2: 543. 1949.

The vacuum pulsations on a milker are passed through a sealed can. The milk from the teat cups enters this can and then is removed automatically through an outlet attached to a container within the can. Removal is accomplished by having a novel valve in the small container; this valve opens and fills the container when vacuum is applied and then closes and forces the milk out the outlet when air pressure is applied.

R. Whitaker

249. Breaking lead halter for cattle. J. G. LAWRENCE. U. S. Patent 2,457,246. 2 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, 617, 4: 1081. 1948.

A halter suitable for leading cattle consists of a loop which encircles the animal's nose and is held in place by a head strap. The loop is made of 2 curved members, hinged on top and bolted together on the bottom, each member carrying a short post and receiving ring on the top.

R. Whitaker

250. Cattle restraining device. H. FLOHR, JR. U. S. Patent 2,458,582. 4 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, 618, 2: 492. 1949.

This device is placed across a gateway where the animals may enter it one at a time. The animal within the device is restrained between two side members which are drawn together and clamped. The holder then may be swung to a horizontal position to facilitate surgical work, de-horning or branding.

R. Whitaker

251. Bull halter and controller. H. MASBRUCH. (Assigned to Russell Mfg. Co.) U. S. Patent 2,458,717. 2 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, 618, 2: 526. 1949.

The novel feature of this halter is a pair of blinders which cover the eyes of the animal allowing forward sight if the head is held high and preventing forward sight if the head is lowered for charging.

R. Whitaker

252. A study of calf-raising facilities in the State of Washington. G. W. SCOTT, JR., R. E. ERB, A. O. SHAW, R. O. GILDEN AND W. E. MATSON. State College of Wash., Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 70. 1948.

Calves were raised satisfactorily in an open pen-tine shed during the winter of 1947-48. Radiant

heating units were unsatisfactory as substitutes for straw bedding for calves.

Facilities available for calf raising in the state of Washington are summarized.

N. L. Jacobson

ICE CREAM

C. D. DAHLE, SECTION EDITOR

253. Ice cream packer. L. J. GILCREST. U. S. Patent 2,457,541. 2 claims. Dec. 28, 1948. Official Gaz. U. S. Pat. Office, 617, 4: 1154. 1948.

A cylindrically-shaped utensil for packing retail packages of ice cream is described. It is equipped with a handle to push the device down into ice cream and a piston which ejects the ice cream from the cylinder when pressed down by a handle which is attached to the piston.

R. Whitaker

254. Arden's diced cream. ANONYMOUS. Ice Cream Trade J., 45, 1: 36. Jan., 1949.

A new package for individual servings of ice cream and a filling machine has been developed. The carton, made of light, waxed paperboard, has a capacity of 3.5 fl. oz. and forms a 2-in. cube of ice cream. The cartons are set up, filled with semi-frozen ice cream, closed by the machine and moved on a conveyor to a hardening room. Freezing is completed in 35 to 40 min., after which they are packed by hand in a heavy chip-board box for distribution to stores. New glassware also has been developed for use in dispensing this new form of ice cream. Quicker, more sanitary dispensing without dipping loss are some of the advantages claimed for the new package.

W. H. Martin

255. 1948 gallonage expected to top 550 million gallons. ANONYMOUS. Ice Cream Trade J., 44, 12: 42, 68. Dec., 1948.

On the basis of U.S.D.A. reports for the first 10 mo. of 1948, which indicate a drop of 11% from the same period in 1947, the gallonage for the entire year will be about 550 million.

W. H. Martin

Also see abs. no. 231, 236, 237, 238.

MILK

P. H. TRACY, SECTION EDITOR

256. Cream dispenser. J. P. JONES. (Assigned to Dairy Specialties Co.) U. S. Patent 2,459,130. 6 claims. Jan. 11, 1949. Official Gaz. U. S. Pat. Office, 618, 2: 633. 1949.

Two holes are provided in the side of conven-

tional paper milk bottles, one at the lower cream level and one at the upper cream level. The holes are closed by a flap which may be swung downward to uncover the upper or upper and lower hole, for removal of milk or cream.

R. Whitaker

257. Milk container. J. P. JONES. (Assigned to Dairy Specialties, Inc.) U. S. Patent 2,457,822. 4 claims. Jan. 4, 1949. Official Gaz. U. S. Pat. Office, 618, 1: 140. 1949.

A square-shaped paper milk bottle is described which employs a perforated shelf located at the level of the bottom of the cream line which facilitates removal of the cream through a wall opening sealed with a partially severed closure tongue.

R. Whitaker

258. Some aspects of pasteurization in a market milk plant. A. G. F. ITZEROTT. Australian J. Dairy Technol. 3, 3: 91-95. July-Sept., 1948.

The effects of low-temperature, holding (LTH) and high-temperature, short-time (HTST) methods of pasteurizing milk on cream line and on bacterial destruction were compared. The temperature and time relationship was 145° F. for 30 min. for the LTH method and 162° F. for approximately 32 sec. for the HTST method. No serious reduction in cream line was observed using the LTH method unless the temperature exceeded 148° F. or the holding period exceeded 45 min. The HTST method likewise had little effect on cream line when measured immediately after pasteurization and cooling. Temperatures above 162° F. (presumably with 32 sec. holding) materially reduced the cream line. Storage of milk after HTST pasteurization for 5, 8.5 and 16 hr., with subsequent agitation prior to bottling resulted in a cream line reduction of 25, 36 and 45 to 50%, respectively. With LTH pasteurization, cream line reduction after similar treatment did not exceed 10%. Plate counts of pasteurized milk, using Difco tryptone-glucose-extract medium with incubation at 37° C. for 48 hr., were obtained at intervals during 1 mo. before and 1 mo. after a change from LTH to HTST pasteurization. During the period of LTH pasteurization, bacterial counts were somewhat lower than those obtained during the HTST pasteurization period. Direct comparisons between the two systems of pasteurization using a split lot of the same milk were not made. Counts obtained after "laboratory pasteurization" agreed with counts obtained after LTH pasteurization but not with counts after HTST pasteurization. In accounting for the differences in bacterial destruction by the two methods of pasteurization, the suggestion was made that the physiological condition of the cells may be impor-

tant, in that mature or aged cells may show a greater degree of heat tolerance to the HTST process than to the LTH process.

J. C. Olson, Jr.

Also see abs. no. 220, 223, 227, 228, 229, 232, 235, 239, 240, 241, 259, 260.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

259. Translating dietary allowances into foods and diets. L. A. MAYNARD. Cornell Univ., Ithaca, N. Y. J. Am. Dietetic Assoc., 25, 1: 18-20. Jan., 1949.

The most significant of the changes in the new Recommended Daily Allowances is the increased recommendation for Ca in the adult diet. The mere addition of more milk and green leafy vegetables in the diet is difficult, since many of the food habits of adults would have to be changed, milk supplies in many localities are inadequate to meet present demands and, in some cases, the incomes of families do not permit them to buy more milk. A desirable plan would be to increase the milk supply, not so much through agricultural production as through more efficient use of present supplies, better distribution and consumer education. Low income groups could be aided by extending public funds, now used to care for the sick, for use in preventing illness by providing adequate diets for those unable to obtain them. In addition to providing more of the superior foods, such as milk, dietary improvement can be brought about through an improvement in the nutritional quality of foods now produced, processed and marketed. The establishment of nutritional standards for foods would involve too many difficulties, but the consideration of such an idea would bring before the public the need for superior nutritional quality of available food supplies.

Elizabeth K. Wheeler

260. Milk in human nutrition. C. P. SEGARD, M. C. Wisconsin Alumni Research Foundation, Madison. Milk Plant Monthly, 37, 12: 58-59, 108-109. Dec., 1948.

The author presents a complete discussion of the importance of vitamins, milk solids, minerals and milk sugar in human nutrition. A discussion of the nutritional value of special milk products such as homogenized milk, "Vitamin D Milk" and fat-free milk also are included.

J. A. Meiser, Jr.

261. Increasing the "invisible milk" in the daily food intake. M. B. BALSLEY, Nutrition Consultant. Evaporated Milk Assoc., Chicago,

III. J. Am. Dietetic Assoc., 25, 1: 58-64. Jan., 1949.

Adult diets are in need of revision in view of the present increase in the Recommended Daily Allowances for Ca from 0.8 to 1 g. More milk can be provided by including dishes prepared with milk. There is considerable variation in the amounts of milk used for the same dish in different recipe books. The selection of those recipes using larger quantities of milk and more concentrated sources, such as dried and evaporated milk, can increase considerably the milk consumption by adults. The inclusion of more cheese and ice cream in the diet is recommended. Additional milk may be used on cereals, in bread-making, on vegetables and in soups, main dishes and desserts. The planning of menus to supply regular amounts of milk in these ways can supplement reduced amounts consumed as a beverage by adults.

Elizabeth K. Wheeler

262. Effect of food supplements on growth, reproduction and lactation. P. B. JUNQUEIRA AND B. S. SCHWEIGERT. Texas A. & M. College, College Station. J. Am. Dietetic Assoc., 25, 1: 46-49. Jan., 1949.

A study was made of the adequacy, for growth, reproduction and lactation in the rat, of diets composed of basal mixtures of whole milk powder and whole wheat, supplemented with meat, casein, liver extract and other factors. Supplementing the basal diet with 11.4% of canned beef caused improvement in the performance of the rats in each of these respects. When the basal ration contained 16.7% whole milk powder, the addition of beef increased the average weaning weights of the young rats from 26 to 42 g. and the average number of young born to the female from 8 to 11. When whole milk powder composed 33.3% of the basal ration, the weights of the weanlings increased from 39 to 43 g. upon the addition of the beef. There was no further improvement in the number of young produced. Improvement in the reproductive performance of the animals was attributed to the additional protein and possibly other unknown factors provided by the beef. The addition of casein caused improvement as did the beef, but the liver extract appeared to have no effect.

Elizabeth K. Wheeler

263. Technik des Tierversuchs und seine Bedeutung für die Bewertung von Milchprodukten. (The technic of the animal test and its importance for the evaluation of milk products.) English summary. LACTONE G. M. B. H. Die Milchwissenschaft, 3, 5: 117-125. May, 1948.

Six groups of 10 albino rats in each group were used in determining availability of riboflavin in

dairy products. The basal diet consisted of 24% dry whey, 12% washed casein, 14% butterfat and 50% potato starch. The six diets used were (a) basal diet with dry whey and casein replaced by skim milk (12 ml./animal/d.), (b) basal diet plus fresh whey (12 ml./animal/d.), (c) basal diet only, (d) basal diet with dry whey replaced by roasted whey (held 1.5 hr. at 200° C.), (e) diet d plus 5% Fuller's earth adsorbate (prepared from whey and containing primarily vitamins B₁ and B₂), (f) basal diet with dry whey replaced by corresponding amounts of lactose, protein and minerals. Optimum weight gains were obtained with diet a followed by b, c and d in the order named. The negative results obtained with diet f were due to the absence of vitamins. The slow gains obtained with d and e were due to the harmful effect of roasting upon the proteins in the whey. The Fuller's earth adsorbate of fresh whey could be used as vitamin B₂ source by the rats.

Four groups of three guinea pigs in each group were used to determine the vitamin C content in the Fuller's earth adsorbate of fresh whey. The basal diet consisted of 65% ground oats, 30% skim milk powder, 1% butter, 1% yeast, and hay *ad libitum*. The ground oats, milk powder and hay were exposed to 110° C. for 6 hr. to destroy the vitamin C. The four diets used were: (a) basal diet only, (b) basal diet plus 0.5 mg. ascorbic acid/animal/d., (c) basal diet plus 20% Fuller's earth whey adsorbate and (d) basal diet plus 40% Fuller's earth adsorbate. The absence of vitamin C in Fuller's earth adsorbate was established.

I. Peters

264. Über den biologischen Wert des Eiweisses von Kefirpilzen im Vergleich zum Eiweiss der Milch, verschiedener Hefen und Schimmelpilze, sowie zum Kartoffeleiweiss. (On the biological value of the proteins of kefir fungi in comparison with protein of milk, of various yeasts and molds and of potatoes.) English summary. H. Fink. Die Milchwissenschaft, 3, 5: 125-133. May, 1948.

Albino rats were used in feeding trials to obtain biological values of proteins from yeasts, molds and potatoes and to compare them with milk proteins. The liver symptoms observed in feeding yeast proteins could be corrected in most instances by the addition of 0.2% L-cystine to the diet. By assigning to milk protein a biological value of 100, beer yeast was given 84; straw-prehydrolyzate yeast, 64; beechwood alkaline sulfite yeast, 34; wood sugar yeast, 33; "*Aspergillus oryzae*", 44; "Biosyn", 53; and potato protein, 54. The type of yeast and its environment, such as growth medium, oxygen relationship, temperature, rate of growth, etc., are mentioned as possible factors controlling the biological value of the resulting protein.

The biological value of kefir protein was 90% of the value of milk protein and was the highest value obtained in plant proteins. Additions of cystine to kefir protein were unwarranted, since no liver symptoms were produced in 90-d. feeding trials. Possible reasons for the high biological value of kefir protein are: (a) symbiosis of bacteria and yeast in kefir, (b) slow growth without aeration, (c) abundant supply of organic nitrogen and growth factors. I. Peters

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

265. Sulfamethazine blood concentrations in calves. C. R. SHROEDER, M. WELSH, R. L. BURKHART, AND P. H. LANGER. Lederle Laboratories, Pearl River, N. Y. *Am. J. Vet. Research*, 10, 34: 63-65. Jan., 1949.

Experiments with 2- to 3-mo. old normal calves showed that sulfamethazine accumulated in the blood on a dosage of 1 g./lb. body weight daily. Optimum dosage was 1 g./lb. on subsequent days. The drug also was administered successfully at the same rate in the milk fed the calf and by subcutaneous injection. No evidence of hematuria, inappetence or blood changes was noted.

E. W. Swanson

266. Studies on vehicles for sustaining penicillin levels in the bovine mammary glands. E. J. FOLEY, A. W. STULTZ, S. W. LEE AND J. V. BYRNE. Wallace Laboratories, Princeton, N. J. *Am. J. Vet. Research*, 10, 34: 66-70. Jan., 1949.

The penetration and persistence of single doses of 100,000 units of penicillin in water-in-oil vehicles infused into the udder were studied. Vehicle A, containing mineral oil, water and lanolin derivatives, was slightly superior to an aqueous infusion, retaining 1.5 to 2.0 units of penicillin/ml. of milk at 36 hr., compared to none from the aqueous vehicle. Vehicle B, containing mineral oil, water, lanolin derivatives, propylene glycol and a nonionic wetting agent, persisted in the lactating gland longest, having 0.4 to 4 units of penicillin/ml. of milk at 72 hr. The vehicles were without clinical irritating effects. Dye in aqueous solution and in vehicle B showed in the excised udder that the effect of the water-in-oil emulsion was due to its general diffusion throughout the gland, while the aqueous solution went only slightly beyond the cistern area. E. W. Swanson

267. The effect of lactation upon the ascorbic acid values of whole blood and blood plasma. R. G. THOMAS, R. E. ERB AND A. O. SHAW. State College of Washington, Pullman. *Proc.*

29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. P. 62. 1948.

In a study of both lactating and non-lactating cows of the Holstein, Jersey and Guernsey breeds a correlation, which was highly significant statistically, was observed between blood plasma ascorbic acid levels and pounds of milk produced per day. When the data for the dry cows were excluded, only the Holsteins showed a significant correlation between the level of milk production and blood plasma ascorbic acid.

N. L. Jacobson

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

268. Control of insects and rodents in dairy plants. E. M. SEARLS, Sealtest, Inc., New York, N. Y. *Milk Plant Monthly*, 37, 11: 83. Nov., 1948.

No rodent-control program will be effective unless rodent-proof barriers are constructed in all the openings leading into the building. Once this has been effected, trapping with a clap-type trap baited with foods that the rats like but don't have access to has been very successful. Suggested baits are bacon rind, smoked fish, raw meat, peanut butter, vegetables, bananas and other fruits. Ten mouse traps and ten rat traps should be sufficient for the average-size plant.

Insect control can be effected best using 5% DDT in an odorless kerosene base. Apply this solution to clean surfaces where the insects go to roost or hide. As kerosene evaporates, it will leave dry DDT crystals which are toxic to insects for long periods. This procedure should be repeated every 3 mo.

J. A. Meiser, Jr.

Also see abs. no. 218.

MISCELLANEOUS

269. History of research dealing with problems confronting the dairy industry which have been conducted by the Agricultural Experiment Stations. A. R. CONLEY. Ohio State University. Mimeograph. 219 pp. 1949.

This publication is divided into two parts. The first part lists, by individual experiment stations, those bulletin publications during the interval covered which are concerned with material related directly or indirectly to the different phases of dairying. The second part arranges the publications listed in the first part according to the subject matter treated, utilizing the title of the publication as the basis for indexing.

This is an interesting publication which undoubtedly will be of value to many. However, many stations have published extensively outside

of their bulletin series; at other stations certain workers may have chosen to publish in journal articles. Therefore, because journal articles are not included, this compilation may give considerably less than a true picture of either the program and publications of a particular station or the contributions which an individual has made.

F. E. Nelson

270. Dairy research under the Research and Marketing Act. E. A. MEYER, U.S.D.A. Milk Plant Monthly, 38, 1: 36, 64. Jan., 1949.

Increased efforts are being made under the Research and Marketing Act to promote the complete utilization of milk and its by-products, including such projects as the use of cheese whey in sherbets as the sole source of milk solids and the use of high grade dry buttermilk solids in ice cream. Economic studies relating to the seasonality of milk prices and the function of co-operatives in the development of milk-pricing plans and methods are being conducted, as are studies of the yields of a unit quantity of milk. In the production field, intensive experiments are being conducted to develop new strains of dairy cattle for hot and humid climates and for owners of small dairy herds.

J. A. Meiser, Jr.

271. Dependability of food judges as indicated by an analysis of scores of a food-tasting panel. ANDREA OVERMAN, Oregon Agr. Expt. Sta., AND JEROME C. R. LI, Oregon State College, Corvallis. Food Research, 13, 6: 441. Nov.-Dec., 1948.

A method of determining the reliability of flavor judgments by members of a food-tasting panel is described and discussed. In the example presented, ten judges scored ten replicate lots containing five food samples having definite flavor variations. The results were analyzed in 2 ways. The score ranges, the number of duplicated judgments and the absolute deviations from the means revealed some of the more obvious differences among the judges. Analysis of variance was used to measure the consistency and the discriminating abilities of the judges. A high ability to recognize differences in samples, together with a low variability in duplicating judgments, are stated as being the marks of a good judge. F. J. Doan

272. Significance in triangular taste tests. E. B. ROESSLER, J. WARREN AND J. F. GUYMON. Univ. of Calif., Davis. Food Research, 13, 6: 503. Nov.-Dec., 1948.

The "triangular" or odd-sample method of distinguishing small differences in flavor between two food samples is described. The samples are presented in groups of three, two in each group being identical. Six orders of presentation are possible: AAB, ABA, BAA, BBA, BAB, ABB. When differences are questionable each taster should be served the samples in all six sequences. The results lend themselves to statistical analysis and a table showing the number of correct answers required to establish significant differentiation is presented. F. J. Doan

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEWS

273. Milk Industry Foundation laboratory manual. Methods of analysis of milk and its products. 2nd ed. Milk Industry Foundation, 1001 Fifteenth St., N. W., Washington 5, D. C. 629 pp. \$15.00. 1949.

This is the second edition of the manual first published in 1933 by the International Association of Milk Dealers. Certain tests have been repeated in the new edition with present accepted modifications. Many new tests have been added in line with recent advances in research and technology. While emphasis is placed upon milk tests, methods are included for almost every dairy product and for certain non-dairy products as well. The form of presentation of subject matter is the same as in the first edition.

The new edition is divided into 8 parts: the milk laboratory; microbiological control of dairy products; chemical control methods for dairy products; physical control methods for dairy products; miscellaneous and special tests of dairy products; microbiological, chemical and physical tests for non-dairy products; preparation of media and reagents; and appendix. Over 175 different tests are described in sufficient detail to permit their performance in the laboratory with little or no additional reference work. Original sources of information are cited liberally. Of particular value is the suggested schedule of routine laboratory procedure, describing the sampling and frequency of testing. This manual should provide an excellent handbook for the dairy plant laboratory technician, the governmental regulatory laboratory and the teacher.

D. J. Hankinson

274. Pregnancy diagnosis tests: a review. A. T. COWIE. 283 pp. 15 shillings. Joint Publication No. 13. Commonwealth Bureau of Animal Breeding and Genetics, Dairy Science, and Animal Health, Penglais, Aberystwyth, Great Britain. 1948.

This book with nearly 2,000 references is an excellent compilation of the literature on various methods of pregnancy diagnosis in women and

domestic animals. The first part of the book deals with clinical methods of diagnosis in domestic animals; clinical methods for women purposely are omitted. Four chapters are devoted to tests dependent upon the presence in body fluids of gonadotropins, estrogens, progestogens and pregnandiol, and other hormones. Two more chapters deal with tests involving enzymic and other biochemical investigations of body fluids and tissues. Chapters concerning tests based on physiological and immunological phenomena and physical properties of body fluids and tissues make up the latter part of the book. The author has placed his evaluation on the methods for the different species concerned. By necessity the references discussed are treated briefly. This book should prove to be of value to practitioners and investigators in the field of human and animal reproduction as well as to many others in related fields.

N. I. VanDemark

275. The chemistry and manufacture of Indian Dairy Products. K. S. RANGAPPA AND K. T. ACHAYA. 189 pp. \$3.00. The Bangalore Press, Bangalore, India. 1948.

The first part of the book deals with the general composition of the Indian milks (from cow, buffalo and goat), breeds of animals and the bacteriological aspects of production and storage. A brief reference has been made to some of the local milk products. Suitable comparative data of the European conditions have been presented. The preparation of butter from the cultured milk has been treated in the second part. Of special interest is the third part, comprising almost half of the book, dealing with the production and the chemical aspects of Ghee, a heat-treated, clarified butterfat with remarkable keeping qualities under warm storage conditions.

The authors have drawn heavily from the Indian workers in the field and the extensive references to the original papers enhance the value of the book. The printing also is satisfactory. The authors deserve to be complimented for a clear and critical presentation of their material.

A. T. Dudani

276. **Odors. Physiology and control.** CAREY P. McCORD, The Industrial Health Conservancy Labs., and William N. Witheridge, General Motors Corp., Detroit. McGraw-Hill Book Co., Inc., New York, N. Y. 1949.

This is the first full-length technical book to appear in the United States that deals completely with the subject of odors, particularly those odors which are classified as offensive. It is a summary of the latest knowledge of the perception, measurement, classification, control and elimination of odors and an appraisal of the significance of odors in relation to health, emotional life, economics and related legal problems.

The physiology, chemistry and psychology of odors are given complete consideration. Then classification, measurement and detection with various methods of control and abatement follow. Finally, how to make an odor survey and legal aspects of odor nuisances complete the coverage. One learns that "Osmics" is the name of the field of science concerned with the sense of smell and that the thousands of materials (including milk and milk products) that lead to the sensation of odor are termed "osmics". Some of the osmics encountered in the dairy industry cannot be designated in the category of "pleasant". This book should be a shelf companion to Crocker's *Flavor*, McGraw-Hill. 1945. L. M. Dorsey

BUTTER

O. F. HUNZIKER, SECTION EDITOR

277. **Pasteurizing and cooling cream and controlling butter defects.** E. J. FERGUSON. Can. Dairy Ice Cream J., 27, 9: 90-92. Sept., 1948.

The practice of cooling quickly, when a surface cooler is used, to 90° F. (where the fat begins to solidify), followed by slower cooling, is the first step toward control of body and texture defects in butter. The correct churning temperature is that which will churn cream exhaustively in 45-50 min., developing granules between the sizes of peas and walnuts for cream testing between 35 and 40% butterfat. Cold washing of the butter granules at about 5 to 10° F. below churning temperature is the most important step in the control of body and texture defects. The butter should be stored at 40° F.

H. Pyenson

278. **Aluminum foil for packaging print butter.** A. H. WHITE. Can. Dairy Ice Cream J., 27, 10: 27-31. Oct., 1948.

Aluminum foil wraps for print butter as compared to parchment give greater protection to flavor quality, prevent the absorption of other

food odors and flavors and eliminate oxidative defects due to light exposure. They minimize loss of weight due to evaporation of moisture from butter surfaces and thus maintain uniform color. The aluminum foil wraps can be used in automatic packaging equipment and make an attractive tightly-wrapped package.

H. Pyenson

279. **Spezifisches Gewicht und Luftgehalt der Butter. (Specific gravity and air content of butter).** English Summary. M. E. SCHULZ. Die Milchwissenschaft, 3, 7: 196-201. July, 1948.

Incorporation of air into butter during manufacture results in larger volume, lower specific gravity, seams and dull, lighter color (in Alpha butter).

A rapid method for the determination of the specific gravity of butter consists in immersing 3 to 4 cm. plugs of butter from a trier into water-alcohol mixtures ranging in specific gravity from 0.85 to 0.95. A minimum of 3 plugs per sample should be examined. Most samples of Alpha butter examined were free of air, whereas churn butter contained from 3 to 5% air by volume and Fritz butter 5 to 10%. I. Peters

280. **Die Eiweisstoffe der Butter. (Proteinaceous substances in butter).** English summary. R. NESENI. Die Milchwissenschaft, 3, 7: 190-193. July, 1948.

Butter samples from Sudeten German dairies were analyzed for (a) total protein, (b) casein, (c) albumin and (d) residual nitrogen by Almen's tannic acid reagent. The values obtained on 63 samples of "Trade Mark" butter varied in *a* from 0.39 to 0.831% (av. 0.518%), in *b* from 0.332 to 0.769% (av. 0.477%), and in *c* from 0.011 to 0.042% (av. 0.024%). The values obtained on 46 samples of "Excellent" butter varied in *a* from 0.166 to 1.31% (av. 0.554%), in *b* from 0.121 to 1.02% (av. 0.488%), and in *c* from 0.013 to 0.055% (av. 0.026%). Analysis of the 109 butter samples after holding for 3 mo. at 8 to 9° C. did not result in a shift of values between the individual protein fractions.

I. Peters

Also see abs. no. 275, 325, 326, 334, 341, 389, 413.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

281. **Control of the moisture content and "body-firmness" of cheddar cheese.** H. R. WHITEHEAD, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Research, 15, 3: 387-397. May, 1948.

Cheesemaking experiments were carried out to investigate the moisture-retaining characteristics of curd made from milks of varying fat and casein content. Milk fat helps to retain moisture in a cheese curd. The higher the proportion of fat present the more drastic the treatment required in the cheese vat to reduce the moisture content of the finished cheese to the desired level. Curd formed from milk of a low-casein content (from Friesian cows) retains moisture in the cheesemaking process more tenaciously than curd from milk of a high-casein content (from Jersey cows). The two effects tend to neutralize each other, but the "casein effect" is usually more powerful than the "fat effect". It is not possible to conclude from the evidence available at present whether the "casein effect" is quantitative only, more moisture having to be removed from a low-casein curd, or whether there also is a qualitative difference between the caseins in Jersey and Friesian milk with an associated difference in moisture-retaining capacity. E. L. Thomas

282. Flavor development in Cheddar cheese made from pasteurized milk. K. V. KOSIKOWSKY AND A. C. DAHLBERG. *Can. Dairy Ice Cream J.*, 27, 10: 70, 74. Oct., 1948.

A popular article based upon J. Dairy Sci., 31: 275-284; 285-292; 293-303; 305-314. 1948.

283. The problem of bitter flavor in Cheddar cheese. E. G. HOOD AND C. A. GIBSON. *Can. Dairy Ice Cream J.*, 27, 11: 45-47. Nov., 1948.

Contributing factors in causing bitterness in Cheddar cheese are acidity, temperature and salt content. The two main factors are pasteurization and lipase activity. Bitterness is intensified by higher pasteurization temperatures and higher storage temperatures. H. Pyenson

284. A comparison of the yields of Cheddar cheese. O. R. IRVINE, L. R. BRYANT, D. C. HILL AND W. H. SPROULE. *Can. Dairy Ice Cream J.*, 27, 11: 49-51. Nov., 1948.

A comparison was made of the yields of cheese/100 lb. of milk from raw milk, from milk pasteurized at 143° F. for 30 min. and from milk pasteurized at 161° F. for 16 sec. The influence of variations in moisture content of the cheese was eliminated by calculating yields on the basis of a 35% moisture content. The mean yields secured on 9 comparative trials were as follows: raw milk, 9.84; holder pasteurized, 10.02; and high short-time pasteurized, 10.01. The high temperature method retained a higher proportion of the butterfat in the cheese than the raw milk. Holder pasteurized milk gave a mean rate of retention intermediate between the

other two. The mean fat content of the whey was significantly lower for the vats of high short-time pasteurized milk than for either raw or holder pasteurized milk. H. Pyenson

285. Cutting large cheese for retail sale. OWEN R. IRVINE. *Can. Dairy Ice Cream J.*, 27, 12: 27-29. Dec., 1948.

In Canada a large 15-in. Cheddar is a standard size cheese for both export and domestic trade. A hydraulic cutter fitted with a special frame is used for cutting large cheese into 18 wedge-shaped pieces. A wire bow is used to slice off a layer of 18 prints. The wedge-shaped prints then have the point trimmed off and the weight checked and then are wrapped in transparent film. Ten 1-lb. prints are boxed so that the wedges form a rectangle. H. Pyenson

286. Future possibilities for the cheese industry. E. W. GAUMNITZ. *Can. Dairy Ice Cream J.*, 27, 9: 54-58. Sept., 1948.

Some of the factors noted in the cheese industry are as follows: (a) production of all cheese has increased steadily, (b) cheese now is produced in practically every state, (c) the government purchases 400 million lb. of cheese annually on the basis of grade, (d) manufacturing methods have been improved generally, (e) extensive use of cheese by the armed forces popularized cheese, (f) the position of cheese was enhanced by putting it in the category of meat, (g) the reduction of imports helped the development of competing domestic varieties, (h) consumer-type packages for American types of cheese have been developed, (i) more varieties of cheese of better quality have been put in packages more acceptable to consumers, (j) the per capita consumption of cheese has increased steadily, particularly in the last few years.

Some of the problems that have become apparent in the past few years are: (a) making of cheese from pasteurized milk (b) more stringent regulations with reference to waste disposal, (c) increased attention to extraneous matter and (d) more efficient utilization of whey.

H. Pyenson

287. Storage of cottage cheese. H. S. WILLARD, Ohio State Univ., Columbus. *Milk Plant Monthly*, 38, 3: 87, 92. Mar., 1949.

Several methods of manufacturing and storing cottage cheese are discussed. Advantages and disadvantages of the different processes are listed. J. A. Meiser, Jr.

Also see abs. no. 297, 301, 307, 308, 330, 333, 349, 389.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

288. The bacteriological quality of British spray-dried milk powder. CONSTANCE HIGGINSBOTTOM, Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland. *J. Dairy Research*, 15, 3: 277-279. May, 1948.

Plate counts on weekly samples of spray-dried milk obtained from 8 plants in Great Britain during the years 1942 and 1943 are presented. A definite improvement in the bacteriological quality of the milk powder was noted in 1943 after the introduction of a preheating temp. of 190° F. Prior to this time a preheating temp. of 165° F. had been used in all spray-drying plants included in this report. E. L. Thomas

289. Keeping quality of dried milk and milk products. P. H. TRAGY.. Cherry-Burrell Circle, pp. 3-5, 22. Mar.-April, 1949.

Present knowledge concerning dried whole milk is summarized in some detail with a number of references to the literature. Dried whey also is discussed. F. E. Nelson

290. Deterioration on storage of dried skim milk. Part I. Introduction. KATHLEEN M. HENRY, S. K. KON, C. H. LEA AND J. C. D. WHITE. **Part II. Preparation, packing and storage of the experimental powders.** C. H. LEA AND J. C. D. WHITE. **Part III. Physical, chemical and palatability changes in the stored powders.** C. H. LEA AND J. C. D. WHITE. **Part IV. Changes in the biological value of the proteins.** KATHLEEN M. HENRY AND S. K. KON. **Part V. Microbiological assay of "essential" amino acids.** KATHLEEN M. HENRY, S. K. KON, C. H. LEA AND J. C. D. WHITE. **Part VI. General discussion and appendix.** KATHLEEN M. HENRY, S. K. KON, National Institute for Research in Dairying, Reading, England, C. H. LEA, Low Temperature Station for Research in Biochemistry and Biophysics, Cambridge, England, AND J. C. D. WHITE, Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland. *J. Dairy Research*, 15, 3: 292-363. May, 1948.

This is a detailed report of the results of a large-scale experiment conducted by the three co-operating research institutes to inquire fully into the changes occurring during storage of dried skim milk.

Little physical or chemical change was observed in the powders with moisture contents of 3 and 5%, except in palatability and gas exchange at the higher storage temperatures. The

high moisture powder (7.6%) rapidly became unpalatable, discolored and insoluble. Its pH, free amino-nitrogen and soluble lactose content fell, whereas the amount of sugar attached to the protein and the reducing power towards ferricyanide increased. Oxygen was absorbed and CO₂ produced. It was concluded that the major cause of deterioration in powder of high moisture content, particularly at high storage temp., is a reaction involving the free amino-groups of the milk protein, which consist mainly of the *ε*-amino-groups of the lysine residues. The primary reaction appears to be between the protein amino-groups and the potential aldehyde group of reducing sugar. The reaction takes place in at least two stages, the primary combination resulting in neither discoloration nor loss of solubility, which follows only as a result of secondary changes, which are not understood fully. The temperature coefficient of the formation (and degradation) of the protein-sugar complex is high (at least 6), and moisture contents which can be tolerated under moderate temperatures for long periods will be unsatisfactory at high temperatures. Physical and chemical properties which depended essentially on the protein-sugar reaction were influenced only slightly by the presence of oxygen.

The protein-sugar reaction results in "heated" or "caramelized" flavors in the gas-packed powders and "stale" and "gluey" flavors in the air-packed powders. Evidence was obtained of an oxidative reaction, independent of the protein-sugar change, which produces an off-flavor in powders stored for long periods at moisture contents too low for the protein-sugar reaction to occur. It is believed that the small amount of fat present is involved. The above factors indicate a decided advantage for gas-packing under all conditions.

Crystallization of lactose, which occurred only in the powder of highest moisture (7.6%), increases the activity of the residual water in the sealed container and further accelerates deterioration.

The biological value of the proteins of dried skim milk of 7.6% moisture content decreased progressively during storage in air at 37° C. from a value of 86.9 for the first 8 d. to 65.9 after 85 d. True digestibility of this powder did not alter after 1 mo. of storage, but a significant decrease of 5-6% occurred by the end of 2 mo., with little change thereafter.

At the lower storage temperature of 28.5° C. the biological value and true digestibility of the protein at the end of 6 mo. was comparable to those of a sample stored for 1 mo. at 37° C., indicating a six-fold difference in the rate of change.

Powder with a 5% moisture content showed an unchanged biological value of its proteins after 6 mo. storage at 37° C., though the true digestibility of the proteins decreased significantly by 4%. Dried skim milks of lower moisture content were not examined, as chemical tests showed they suffered little deterioration under any conditions of storage.

Microbiological tests of fresh and stored dried skim milk showed a definite apparent loss of lysine in deteriorated powder, the loss being greater when measured after enzymic than after acid hydrolysis. The maximum deficiency of lysine was about 40% of the original content of this "essential" amino acid. A slight loss of histidine also was probable, and of arginine and methionine possible, but the reproducibility of the methods was not sufficient to establish these with certainty. Restoration of the approximate original biological value of the proteins in a sample stored for 60 d. at 37° C. in nitrogen was obtained by the addition of 1.25% lysine. An increase in the true digestibility of the proteins and in protein efficiency also was observed, but the values fell short of those obtained for the control milk. The addition of histidine as well as lysine caused a further slight improvement in the protein efficiency of a sample of deteriorated milk which had been stored for 60 d. at 37° C. in air, but arginine was without effect. The authors emphasize that it seems likely that under all conditions of storage skim milk powder will become unpalatable before it suffers any appreciable loss in the nutritive value of its proteins.

A discussion is presented in which the conclusions reached from the various approaches to the problem are considered in relation to one another.

E. L. Thomas

291. The manufacture of powdered cream. P. H. TRACY. *Can. Dairy Ice Cream J.*, 27, 11: 52-53. Nov., 1948.

As with fluid cream, reconstituted cream standardized to 30-34% butterfat whipped by aeration produced a better whipped product than that containing less butterfat. The addition of added m.s.n.f. was beneficial up to approximately 8%. Emulsifying agents such as sorbitan monostearate aid in obtaining overrun, good body and texture, and dry appearance of the product whipped by aeration. There were no noticeable differences in whipping properties of the reconstituted creams sprayed from various size nozzles. High spray pressures were detrimental to the whipping ability of the reconstituted cream. Inert gases delayed oxidized flavor development. The addition of anti-oxidants like N.D.G.A., gallic acid and Vitamin to powdered cream, especially when nitrogen

packed, increased the shelf life of the powder.

H. Pyenson

292. Concentrated milk and the ice cream industry. D. B. GOODWILLIE. *Can. Dairy Ice Cream J.*, 28, 1: 27, 29, 72. Jan., 1949.

The concentrated milks discussed are plain condensed milk, dry whole milk, dry skim milk, dry buttermilk, dry ice cream mix and dry cream for whipping.

H. Pyenson

293. Problems of experimental condensing and spray drying. H. SHIPSTEAD AND R. L. PERRY, Univ. of California, Davis. *Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc.* Pp. 90-101. 1948.

A laboratory spray drier and a small milk-condensing unit are described. Drawings of the units are shown and the results of two trials with the drier are presented.

H. B. Naylor

294. Untersuchungsmethoden für Milone. (Methods for analysis of Milone.) English summary. K. KUMETAT. *Die Milchwissenschaft*, 3, 7: 193-196 July, 1948.

Analysis of the alcoholic whey beverage "Milone" resulted in values as follows: specific gravity, 1.0047; alcohol, 0.82% by volume; total acidity (expressed as lactic acid), 0.63% volatile acids (expressed as acetic acid), 0.083%; ash, 0.31%; dry matter, 1.25%; lactose, 0.18% and protein, 0.19%. Analytical methods are given.

I. Peters

Also see abs. no. 304, 305, 316, 319, 329, 399.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

295. Inhibitory strains of lactic streptococci and their significance in the selection of cultures for starter. MARGERY HOYLE AND AGNES A. NICHOLS, Univ. of Reading, Reading, England. *J. Dairy Research*, 15, 3: 398-408. May, 1948.

From 56 "wild" strains isolated from sour milk for use as starter strains, 19 (34%) were found to be inhibitory. Since the inhibitory strains came from eight samples of milk it is likely that some of the strains may have been identical. Fifty-nine (27%) inhibitory strains from 220 "cultivated" strains of *Streptococcus cremoris* were obtained out of a large collection of starters. Several of these inhibitory strains also may have been similar since they were obtained from only 13 starters. All of the inhibitory strains from starter were *S. cremoris* while those from "wild" sources were classified as *Streptococcus lactis*, although several of the latter gave atypical reactions to some differential tests.

The importance of testing individual strains of lactic streptococci for inhibitory properties is emphasized, especially when strains are prepared separately to be mixed later. The importance of this fact in connection with slow acid development in cheesemaking is considered. Methods are described for determining the concentration of inhibitory substance produced and the effect of these inhibitory strains on a wide selection of lactic streptococci.

E. L. Thomas

296. The identity of streptococci from starter and of streptococci, suitable for use as starter, isolated from sour milk. AGNES A. NICHOLS AND MARGERY HOYLE, National Institute for Research in Dairying, Reading, England. *J. Dairy Research*, 15, 3: 409-416. May, 1948.

277 strains of lactic streptococci isolated from commercial starters and 72 strains from souring milk, suitable for cheesemaking, were identified. All the starter strains examined were *Streptococcus cremoris* and those (with two possible exceptions) from the sour milk were *Streptococcus lactis*. Since the strains from each source were so similar, the information obtained did not help in the selection of the strains within each group most suitable for starter. Action in litmus milk, hydrolysis of arginine, and ability to grow in litmus milk at 40° C. (104° F.) usually will be sufficient to differentiate clearly between *S. lactis* and *S. cremoris*.

E. L. Thomas

297. The problem of bacteriophage in relation to cheese starters. C. C. PROUTY. *Can. Dairy Ice Cream J.*, 27, 9: 46-48. Sept., 1948.

Bacteriophage is the major cause of slow starter. Bacteriophage is a virus living as a parasite upon the bacterial cell. Bacteriophage are not destroyed until a temperature of 158° F. and an exposure period of 15-30 min. is obtained. They also are more resistant than bacteria to drying, storage and action of acid and alkalis. Bacteriophage can be destroyed by 500 p.p.m. chlorine concentration in 1 min., and by hypochlorite mists over a temperature range of 30-80° F. if the room has over 50% humidity and the hypochlorite has a concentration as little as 0.02 to 0.003 p.p.m. Generally a bacteriophage strain will be active against one specific strain of lactic acid bacteria. With the appearance of slow starter in a cheese factory, a new starter carrying a different or several different strains is placed in use. Starter rotation may help to control bacteriophage by avoiding the building up of a high concentration of any one bacteriophage strain. Resistant strains of lactic acid bacteria can be developed from a bacteriophage-susceptible strain by prolonged incubation of the culture.

The initial source of bacteriophage in a cheese factory is difficult to determine. Once it is established in the cheese factory, it will develop rapidly in the cheese vats as it survives pasteurization. The whey separator and whey storage vats should be located in a separate room from the cheese vats, as the whey may seed succeeding vats of milk with bacteriophage. In New Zealand, workers advocate the use of a separate building in which to propagate the cheese starter for daily use to prevent contamination from bacteriophage.

H. Pyenson

298. Studies in the bacteriology of milk. I. The streptococci of milk. Y. ABD-EL-MALEK AND T. GIBSON, College of Agriculture, Edinburgh, Scotland. *J. Dairy Research*, 15, 3: 233-248. May, 1948.

This is the first of a series of papers in which the authors report upon investigations designed to provide a clearer picture of the composition of the bacterial populations in different types of milk.

The present paper deals with the frequency of occurrence of individual species of streptococci in raw and pasteurized milks of varying purity. Samples were examined in the raw state and also after laboratory pasteurization at 63° C. (145.4° F.) for 30 min. Some of the pasteurized samples were held for further observation at various temperatures between 10 and 22° C. until a taint and other signs of bacterial action had appeared. Details of methods employed for the identification of species are given.

Twenty-two out of 23 samples of raw milk yielded streptococci. Of the 22 positive samples, *Streptococcus kefir* was detected in 19 samples, *Streptococcus lactis* in 17, mastitis streptococci in 16, *Streptococcus cremoris* in 10, *Streptococcus faecalis* in 7, *Streptococcus citrovorus* in 3, and *Streptococcus bovis* in 2 samples.

Thirty-three out of 54 samples of fresh pasteurized milk yielded streptococci. *Streptococcus thermophilus* was detected in 28 of the samples, *S. bovis* in 25, *S. faecalis* in 6, and *S. kefir* in 5. Pasteurized milk held at 10-22° C. until it became tainted yielded *S. kefir* and *S. faecalis* at the lower temperatures and, at the higher temperatures, *S. bovis* and *S. thermophilus*.

The authors report that a notable feature of the results is the prevalence of *S. kefir*. This organism was isolated from all classes of raw milk with greater frequency than *S. lactis* and although it suffered considerable destruction during pasteurization, it frequently became the dominant species in pasteurized milk stored at 10-22° C.

The streptococci occurring in raw and pasteurized milk were placed in five main groups by test-

ing for the hydrolysis of arginine, growth at 45° C. and at 8–12° C., survival in milk at 63° C. for 30 min. (measured by a semi-quantitative plating method), action on litmus milk, and ability to form CO₂ from glucose (tested by a cultural method). The five groups thus distinguished were: (1) organisms of bovine mastitis; (2) *S. bovis* and *S. thermophilus*; (3) *S. lactis* and *S. cremoris*; (4) *S. faecalis* and its varieties and (5) heterofermentative streptococci (*S. kefir* and *S. citrovorus* predominating). F. L. Thomas

299. Studies in the bacteriology of milk. II. The staphylococci and micrococci of milk. Y. ABD-EL-MALEK AND T. GIBSON, College of Agriculture, Edinburgh, Scotland. J. Dairy Research, 15, 3: 249–260. May, 1948.

A total of 598 cultures of staphylococci and micrococci from milk and dairy equipment were studied in detail. These were isolated as follows: 248 from the cow's udder (milk drawn aseptically), 76 from raw milk, 242 from pasteurized milk and 32 from pasteurized rinsings of dairy equipment. For purposes of comparison, 201 additional cultures from other known sources also were examined. Details of the laboratory methods used are given, as well as a critical discussion of the limitations of the various criteria for identifying species.

As a result of the present study, the authors have devised a classification of representatives of the *Staphylococcus-Micrococcus* complex occurring in milk. According to their general character the strains could be arranged in a virtually continuous series. At one extreme is placed the pathogenic staphylococcus and at the other a thermophilic saprophyte. The series consists of three main groups as follows:

"(1) The staphylococcus group in which the organisms are sugar fermenters and relatively sensitive to heat. They are, mainly at least, parasites of the animal body. Four subgroups are distinguishable. In three ammonia is formed from arginine, and in two acetoin is formed from glucose. A test for acetoin production defines those mannitol fermenters which do not produce coagulase.

"(2) An intermediate group in which the organisms are obligate aerobes and do not produce acid from sugars.

"(3) The dairy micrococci, a group of thermophilic sugar fermenters which occur frequently on dairy equipment and in pasteurized milk. The group comprises two species conforming to *Micrococcus luteus* Cohn *emend.* Lehmann and Neumann, and *Micrococcus varians* (Dyar) Migula." E. L. Thomas

300. The microbacteria. I. Morphological and physiological characteristics. R. N. DOETSCH AND M. J. PELCZAR, JR., Univ. of Maryland, College Park. J. Bact., 56, 1: 37–49. July, 1948.

The history of the classification of the members of the genus is reviewed. Eighteen cultures were studied to observe microscopic, cultural and physiologic characteristics. These included five original isolations made from milk following laboratory pasteurization. Thermal resistance, while valuable for isolation purposes, was not a satisfactory characteristic for classification. A key is presented to describe three species: (1) *Microbacterium* sp., (2) *M. lacticum* and (3) *M. flavum*. D. P. Glick

301. Bacteria in farmer's milk which survive pasteurization and subsequently grow during cheese making. I. ERICHSEN AND N. S. GOLDING, State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. Pp. 110–116. 1948.

Thirty-seven samples of raw milk from different producers were studied to determine the effect of incubation temperature on plate counts made before and after laboratory pasteurization. Standard methods were followed with the exception that cabbage extract was added to the standard agar. The incubation temperatures studied were 30 and 37° C. Incubation temperature had a marked influence on raw milk counts, and on pasteurization efficiency calculated from counts on laboratory pasteurized samples.

The ability of pure cultures isolated from pasteurized milk plates incubated at 30 and at 37° C. to grow in milk incubated at temperatures and times comparable to those used in cheesemaking was determined. About 25% of the 106 cultures tested showed considerable growth under cheese-making conditions. H. B. Naylor

302. Studies on heat resistance. I. Increasing resistance to heat of bacterial spores by selection. FRANKLIN L. DAVIS, JR., AND O. B. WILLIAMS, Univ. of Texas, Austin. J. Bact., 56, 5: 555–559. Nov., 1948.

Using spores of *Bacillus globigii*, it is shown that there is a graded resistance to heat among the spores of a population. By selecting the survivors of heated spore suspensions, an increase in resistance was observed. D. P. Glick

303. Studies on heat resistance. II. Comparison of resistance to heat with resistance to disinfectants. FRANKLIN L. DAVIS, JR., ORVILLE WYSS AND O. B. WILLIAMS, Univ. of Texas, Austin. J. Bact., 56, 5: 561–567. Nov., 1948.

When spores of a culture of *Bacillus globigii* and of a heat-resistant variant derived from the parent culture were plated in parallel in agar media containing various amounts of gentian violet, mercuric chloride or streptomycin, both strains were inhibited similarly by increasing concentrations of the bacteriostatic agents. However, the spores of the heat-resistant strain exhibited greater resistance to the killing action of iodine and of phenol.

D. P. Glick

304. **Dye-reduction tests in the bacteriological examination of dried milk.** CONSTANCE HIGGINBOTTOM, Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland. J. Dairy Research, 15, 3: 280-284. May, 1948.

Results from both the methylene blue-reduction and the resazurin-reduction tests on reconstituted spray-dried milk showed very poor correlation with plate counts at either 37 or 30° C. and with keeping quality.

E. L. Thomas

305. **Bacterial growth in reconstituted spray-dried milk.** CONSTANCE HIGGINBOTTOM, Hannah Dairy Research Institute, Kirkhill, Ayr, Scotland. J. Dairy Research, 15, 3: 285-291. May, 1948.

Over 200 samples of spray-dried milks from 8 British plants were examined for total and spore counts, numbers of beta-haemolytic colonies, molds, yeasts and coliform bacteria. Results are reported for the freshly reconstituted products and also after aging the reconstituted milk for 24 hr. at 15.5° C. and at 22° C. Predominating organisms present in a random selection of samples are described. The relation of the flora of reconstituted milk to its food-poisoning potentialities is discussed briefly.

E. L. Thomas

306. **Carbon assimilation tests for the classification of yeasts.** L. U. WICKERHAM AND K. A. BURTON, Northern Regional Research Laboratory, Peoria, Illinois. J. Bact., 56, 3: 363-371. Sept., 1948.

This is an extension of the senior author's earlier work on fermentation tests and nitrogen assimilation tests. The basic medium is synthetic; it contains vitamins in pure form instead of yeast extract or other crude vitamin carriers. Seventy carbon compounds have been tested on 100 strains of yeasts, representing 22 genera, for preliminary classification of the compounds. These are presented in nine categories of usefulness. The advantages of assimilation tests are emphasized in comparison with the less satisfactory fermentation tests.

D. P. Glick

307. **Physiological reasons for the dominance of *Penicillium roqueforti* in blue veined cheese.**

N. S. GOLDING AND D. D. MILLER, State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. Pp. 122-129. 1948.

Cultures of *Penicillium roqueforti*, *Penicillium expansum* and *Oospora lactis*, cultured on malt agar at 55° F., were compared as to ability to grow in atmospheres of different CO₂ and O₂ content. It was found that *P. roqueforti* was not inhibited by CO₂ until a concentration of over 30% was reached. The other cultures were inhibited measurably at the 10% level. On the other hand, *P. roqueforti* was inhibited to a greater extent than the other cultures when the O₂ content was less than 4%. There was no growth of *P. roqueforti* at O₂ levels below 0.8%, whereas *P. expansum* showed growth at the 0.3% level and *O. lactis* grew at even lower concentrations.

Since the ranges of CO₂ and O₂ concentrations in blue veined cheese are 21.14 to 40.95% and 2.42 to 7.00%, respectively, it was concluded that the reason for the predominance of *P. roqueforti* in blue cheese is due to the high CO₂ content and not to the low O₂ content.

H. B. Naylor

308. **The control of mold.** D. D. MILLER. Can. Dairy Ice Cream J., 27, 9: 60-64. Sept., 1948.

A very low concentration of carbon dioxide may stimulate rather than inhibit mold growth, particularly at a storage temperature of 70-80° F. High concentrations inhibit mold growth especially at low storage temperatures. Probably the best method to inhibit mold growth by use of gas at cool temperatures is to reduce the O₂ present to a minimum and then to add a fairly high concentration of CO₂. In the vacuum canning of cheese, the small amount of O₂ in the can, the CO₂ formed by fermentation in ripening, the conversion of the O₂ in the can to CO₂ by aerobic growth of molds and other organisms all tend to prevent mold growth. The use of CO₂ in the inhibition of molds may be applied to the canning of cheese, and cheese and butter rooms properly built to allow the addition and retention of a given concentration of CO₂.

H. Pyenson

309. **Bacteriological changes during the fermentation of steamed potatoes for silage.** J. L. ETCHHELLS AND I. D. JONES. J. Agr. Research, 78, 1 and 2: 19-31. Jan. 1 and 15, 1949.

Thermophilic, facultative anaerobes were the predominating microorganisms during the fermentation of hot-ensiled steamed potatoes (*Solanum tuberosum*) and were responsible for the developed acidity and resultant preservation of the silage. These bacteria are considered to be

non-gas-producing, acid-forming, spore-bearing rods, which are facultative with respect to oxygen and temperature requirements. These organisms may be classified, according to Bergey *et al.*, as thermophiles belonging in group X of the genus *Bacillus*. H. Pyenson

Also see abs. no. 288, 290, 372, 407.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

310. Investigations on the composition of South African milk. IV. The influence of monthly variations in air temperature and rainfall on the composition of milk. S. BAKALOR, Agricultural Research Institute, Pretoria. *Farming in S. Africa*, **23**, 265: 271-282. April, 1948.

The relationship of air temperature and amount of rainfall to the fat and solids-not-fat content of milk was determined for various areas in South Africa. There was no correlation between mean fat content of milk and air temperature for the same month, but there was an increase in fat content as winter approached and a decrease in fat content as summer approached.

The amount of rainfall had no effect on fat content, but the amount of solids-not-fat increased with an increase in rainfall. There was no apparent relationship between air temperature and solids-not-fat content of milk. F. C. Fountaine

311. Investigations on the composition of South African milk. V. (a) The relationships between the various constituents of milk. S. BAKALOR, Agricultural Research Institute, Pretoria. *Farming in S. Africa*, **23**, 266: 345-354, 356. May, 1948.

Data were taken from analyses for fat, protein, ash, lactose, T. S. and solids-not-fat of 1,608 industrial samples of milk supplied by producers, plus T. S., S. N. F. and fat on 1,200 batches of whole milk. Annual averages of fat and S. N. F. of milk supplied to condenseries over a period of 15-16 yr. also were used. There was no definite relationship between S. N. F. and fat and ash and fat on individual samples, but a definite positive relationship existed between fat and S. N. F. when annual averages were used.

Increased fat % was accompanied by increased protein % and decreased lactose %. There was a variation in the S. N. F. content of groups of milk with fat % constant, the ash content being the most variable between fat-constant samples. There also was a seasonal change in the S. N. F. content of milk, independent of changes in fat content. S. N. F. and lactose were highest in summer; S. N. F. and protein were highest in late summer and autumn; S. N. F., protein and

lactose were lowest in late winter and early spring. There was a positive relationship between protein and fat and a negative relationship between lactose and fat. F. C. Fountaine

312. Investigation on the composition of South African milk. V. (b) The ratios of the percentages of other constituents to the percentage fat in milk. S. BAKALOR, Agricultural Research Institute, Pretoria. *Farming in S. Africa*, **23**, 267: 415-422. June, 1948.

As the % fat increases, a definite narrowing of the ratio of S. N. F., ash, protein and lactose to fat occurs. In all but one area the ratio of S. N. F. to fat was widest in the spring and summer months. The importance of changes in ratios of milk constituents to the industry is discussed. F. C. Fountaine

313. Undersøkelse over melkens aciditet. (Studies concerning milk acidity). English summary. H. DOVLÉ. *Meieriposten*, **37**: 415-419. 1948.

Daily milk samples were obtained from 80 cows. The mean pH was 6.65, with the range from 6.45-6.83, and 90% of the samples were in the range from 6.60-6.74. The mean titratable acidity was 7.10° SH, the range 5.50-8.15° SH, and 74.2% of the samples were in the interval 6.75-7.70° SH. Disease caused marked changes in pH and titratable acidity. Titratable acidity could not be calculated from pH and vice versa. O. M. Ystgaard

314. Observations on change of buffer indices of milk with changes of temperature. L. S. VODAK AND N. P. TARASSUK, Univ. of California, Davis. *Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc.* Pp. 102-109. 1948.

Fresh milk samples were heated to temperatures ranging from 42.5 to 93° C. and held for 30 min. The samples then were cooled rapidly to 4.5° C. and stored at that temperature. Samples of the unheated milk cooled to the same temperature were used as controls. At zero time and after 2, 4, 8, 12 and 24 hr., electro-metric titrations were made on aliquots of each sample. 0.1 N HCl was used to titrate to pH 4.0, and 0.1 N NaOH was used in titrating to pH 8.5. Titration curves were plotted and the buffer indices were computed for eight pH values.

The results showed that buffer indices increased at most pH values when unheated milk was cooled and held at 4.5° C. Heating decreased the buffer indices, the greatest decreases being observed at the highest temperature. However, the changes produced by heating were reversible, as shown by the fact that storage of the

heated samples at 4.5° C. for from 2 to 24 hr. resulted in buffer indices which were nearly identical with those of the control samples.

H. B. Naylor

315. Improved dairy indicator solution. L. R. BRYANT. *Can. Dairy Ice Cream J.*, 27, 8: 31, 48. Aug., 1948.

Dairy indicator solution that has better keeping qualities than phenolphthalein in an alcoholic solution is made by dissolving 1 g. of dry phenolphthalein in 60 ml. of cellosolve (ethylene glycol mono-ethyl ether) or methyl cellosolve and then diluting with 40 ml. of water. This solution retains its proper concentration over a longer period of time than does an alcoholic solution of phenolphthalein.

H. Pyenson

316. Ion exchange applications in milk products. H. E. OTTING, M & R Dietetic Labs., Inc., Columbus, Ohio. *Ind. Eng. Chem.*, 41, 3: 457-459. Mar., 1949.

This paper is one of six which made up a symposium on the application of ion exchange in different industries. Calcium first was removed from milk in 1930 by placing it in contact with greensand. The resulting milk exhibited soft-curd properties. Years of research have resulted in the present process and the use of a hydrated synthetic sodium aluminum silicate. One ft.³ of this ion exchange material will treat 125 gal. of milk per cycle and it now can be used daily for more than a year with little replacement. The process, which dairy plant employees can be trained to carry out, follows: Wash the base-exchange material (upflow) with water at 100° F., to remove residual milk. Circulate (upflow) a wetting agent to remove fat, protein and phosphorus and rinse with water. Pass acidified and buffered sodium chloride (5%) downflow and follow with a water rinse. Circulate downflow a solution of sodium hydroxide and sodium aluminate and wash with water.

Soluble caseinates of improved keeping quality can be made by this process. Development of sandiness in ice cream mixes which contain as much as 15% milk solids-not-fat can be retarded if 3% or more of these solids are ion-exchange-treated solids. The heat stability of evaporated milk can be improved sufficiently to eliminate the need for stabilizing salts if 0.5-2% of the original milk is treated by the mineral-ion exchange process.

B. H. Webb

317. Undersøkelser over noen varianter av Kjeldahls metode til bestemmelse av protein i melk og melkeprodukter. (Investigations of some modifications of the Kjeldahl method for the determination of protein in milk and milk

products.) H. DOVLE. *Meieriposten*, 37: 473-477. 1948.

Heating almost to the boiling point and slow boiling, after raising the boiling point by addition of potassium sulfate (Gunning) were the heating conditions tested: Lower values always were obtained when the digestion was ended immediately after the liquid looked clear. Mountain milk caused trouble even when boiled as much as 1.5 hr. after the liquid looked clear.

Flasks containing 500 ml. were preferred because this size secured better acid condensation and the flasks could be used conveniently as distillation flasks; 750 ml. flasks also were found adequate. If steam distillation were used, 100-300 ml. flasks were adequate.

The recommended procedure for the determination of nitrogen in milk and milk products is as follows: (a) Add 5-8 ml. of milk/Kjeldahl flask; (b) add approx. 1 g. crystalline CuSO₄ (or 0.3-0.5 g. CuO); (c) add H₂SO₄ (3 × the milk volume if gas heating is used or 4 × the milk volume if electrical heating is used); (d) heat slowly until the water has evaporated, then raise the temperature to obtain slow boiling; (e) cool the flasks after 0.5 hr.; (f) add crystalline K₂SO₄ in the amount of 1/2-2/3 as many g. as the no. of ml. of H₂SO₄ added; (g) continue the heating process (slow boiling); (h) cool after approx. 2.5 hr. when the sample turns green; (i) add water. (Precipitation takes place at first, but enough water is added to dissolve the precipitate.) Distillation, etc., are carried out as usual.

O. M. Ystgaard

318. Determination of the free amino-nitrogen of casein and of fresh and deteriorated milk protein by the Van Slyke method. C. H. LEE. *Univ. of Cambridge, Cambridge, England. J. Dairy Research*, 15, 3: 364-368. May, 1948.

The reaction of casein, fresh milk protein and deteriorated milk protein with nitrous acid was followed at 20° C. for 4 hr. in the manometric apparatus of Van Slyke. Simplified procedures are suggested whereby the method can be utilized for investigation of the deterioration of the protein of skim milk powder during storage.

For the investigation of deteriorated spray-dried skim milk powder, the powder being brown in color and of very poor solubility, in the present study, it was first suspended in water, dialysed at 0° C. for 5 d. and freeze-dried. One hundred mg. of the product, which dissolved quite readily in the acetic acid in the reaction chamber, were used for each determination. Diphenyl ether proved inadequate as a defoaming agent but secondary octyl (capryl) alcohol proved satisfactory. Commercial samples of capryl alcohol were

found to vary greatly in purity. The use of one drop of a redistilled fraction boiling within 1° C. of the correct figure was used as standard procedure. For routine examination of deteriorated milk powders, a reaction time of 30 min. was adopted as a standard, the values (mg. free amino N/g. protein N) so obtained being corrected by the addition of 2 units. Since some samples of milk protein appear to reach the linear portion of the reaction/time curve appreciably earlier than others, an error of as much as ± 1 unit may be introduced by this approximation.

E. L. Thomas

319. Separation of proteins from milk products. A. LEVITON. (Assigned to the people of the U. S.). U. S. Patent 2,460,891. 1 claim. Feb. 8, 1949. Official Gaz. U. S. Pat. Office, 619, 2: 431. 1949.

Dried skim milk powder is separated into a protein fraction and a lactose fraction by precipitation of the former by mixing the powder with a 40–62% (by wt.) methanol-water soln. at -16° C. and filtering immediately.

R. Whitaker

320. Reststickstoffgehalt in normaler Kuhmilch. (The residual nitrogen content in normal cow's milk). English summary. R. NESENI AND H. KÖRPRICH. Die Milchwissenschaft, 3, 7: 186–189. July, 1948.

The milk of 11 healthy cows was examined for residual nitrogen content, using Almen's reagent, every 14 d. during the period between September 15, 1943, and July 21, 1944. The extreme range of values fluctuated from 13 to 40 mg. %, with the average being between 27 (± 4.55) and 32 (± 4.8) mg. %. Higher values were obtained at the beginning and end of the lactation period than during the in-between period. Green feed as well as some concentrates tended to increase the residual nitrogen values in milk, whereas dry feeds reduced it. Values above 40 mg. % can be regarded as abnormal and may be considered as symptoms of pathological conditions in the animal of a sub-clinical nature.

I. Peters

321. Process to produce a stabilized protein-formaldehyde dispersion. L. L. MCKINNEY. (Assigned to the people of the U. S.). U. S. Patent, 2,461,070. 6 claims. Feb. 8, 1949. Official Gaz. U. S. Pat. Office, 619, 2: 475. 1949.

Casein is allowed to react with an alkylene oxide in an alkaline medium before hardening with formaldehyde.

R. Whitaker

322. The reaction between milk protein and reducing sugar in the "dry" state. C. H. LEA, University of Cambridge, Cambridge, England. J. Dairy Research, 15, 3: 369–376. May, 1948.

Unheated, fresh milk was separated and dialysed at 0° C. in the presence of a little toluene against repeated changes of distilled water for a total period of 7 d. The solids content was determined and the requisite quantities of glucose, lactose, sucrose and mixtures of these sugars were added to portions of the fluid which then were freeze dried. After equilibration over sulfuric acid to the required moisture content, samples were stored at 37° C. and 55% relative humidity. At intervals samples were examined for free amino and total nitrogen, solubility of the protein and color.

The reducing sugars combined with free amino-groups of the protein, apparently in a 1:1 ratio. The reaction did not proceed to completion, probably owing to difficulty of access of the reactive groups to one another. Only when the sugar-amino reaction occurred did discoloration ensue. Glucose reacted more rapidly with the protein than did lactose, and the complex formed became discolored and insoluble in both cold and hot water much more rapidly. Sucrose and lactose both greatly delayed the onset of glucose-induced insolubility, lactose being the more efficient of the two. They did not prevent discoloration.

The protein alone became insoluble in cold but not in hot water after prolonged storage, but did not discolor. This change was prevented by sucrose. The behavior of lactose was inconsistent, loss of solubility being accelerated in one experiment and retarded in another. It is suggested that the effect of sugars on the development of insolubility in proteins includes (a) acceleration by reason of the reducing group-amino reaction and (b) retardation under the influence of relatively high concentrations of carbohydrate, particularly of disaccharide. Glucose behaves predominantly according to mechanism *a*, sucrose entirely according to mechanism *b* and lactose according to both.

E. L. Thomas

323. The effect of varied concentrations of non-fat dry milk solids on the rate of whey protein denaturation by heat. G. J. KRUEGER, U. S. ASHWORTH AND H. A. BENDIXEN, State College of Washington, Pullman. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. Pp. 82–89. 1948.

Using nonfat dry milk solids spray dried from separated milk which had not been given a pre-heat treatment, a study was made of the effect of temp. on the extent of whey protein denaturation in reconstituted samples varying in milk

solids concentration. Samples reconstituted at the rate of 1, 5, 10, 16, 20, 25 and 30 g. of powder per 100 ml. water were heated to temperatures of 70, 75, 77, 80 and 85° C. and held for 30 min. The undenatured whey protein was determined in each case by saturating the sample with NaCl, filtering off the precipitate and measuring the turbidity of an aliquot of the filtrate after acidification with HCl. At temperatures of 77° C. and below, the extent of whey protein denaturation decreased as the milk solids content was increased. However, at 80 and 85° C., samples containing low levels of milk solids showed abnormally low levels of denatured whey proteins; the authors indicate that this may have been due to the presence of acid-coagulable breakdown products of casein in the filtrates.

H. B. Naylor

324. Manufacture of artificial protein filaments. R. H. K. THOMPSON. (Assigned to Imperial Chemical Industries, Ltd.). U. S. Patent 2,460,372. 12 claims. Feb. 1, 1949. Official Gaz. U. S. Pat. Office, 619, 1: 131. 1949.

A casein solution containing a vegetable globulin is spun as a filament into a formaldehyde bath, then dried at at least 80° C. under tension, followed by contact with boiling water while in the relaxed condition.

R. Whitaker

325. The properties of New Zealand butters and butterfats. I. Iodine, Reichert and saponification values and softening points of monthly samples of butterfats from nine commercial factories over four years. G. A. COX AND F. H. McDOWALL, Dairy Research Institute, Palmerston North, New Zealand. J. Dairy Research, 15, 3: 377-386. May, 1948.

The trend of variation of any one property throughout the season was remarkably uniform, both for different factories in the one season and for any one property in the four seasons. Weighted monthly average iodine values, Reichert values, saponification values and softening points for the butterfat from all factories over 4 yr. were 36.7 (33.8-40.2), 30.4 (25.5-32.3), 229 (225.5-232.7) and 33.1 (32.2-33.7), respectively. The minimum iodine value occurred in midsummer, i.e., at the season of the year when maximum values are reported for northern hemisphere butters. The iodine values for South Island butterfats diverged markedly from those for the North Island butterfats during the winter, i.e., at the time when turnips are fed to cows in the South. In spite of the lower iodine values, the softening points of the South Island butterfats were lower throughout the year. An explanation of the latter observation must await a study of the detailed fatty acid composition

of the butterfats at the different periods of the season.

E. L. Thomas

326. Onderzoek naar de factoren die de samenstelling van het melk vet beïnvloeden. (Investigation into the factors influencing the composition of milk fat.) English, French and German summaries. W. ADRIANI, A. F. TAMSMA, M. P. VOGEL AND J. GROOT. Laboratorium de Coöperatieve Fabriek van Melkproducten, Bedum, Holland. Vakgroep Boterindustrie. The Hague, Holland. 182 pp. + 24 graphs. 1945.

It has been found in practice in Holland that during the pasture period the composition of the milk fats often was such that even with the best manufacturing process the butter still remained too soft, and so the composition of the milk fat was primarily decisive for the consistency of summer butter. There was a large difference in this respect between different parts of Holland, making further study desirable.

As a criterion for the composition of the milk fat, the refractive index was chosen and ample proof given that it may be considered a good indicator.

In the first place, experiments were conducted with cows grazing in the same pasture and the following factors found to show correlation with the composition of the fat: age of the cow, fat production, fat percentage of the milk, ponderosity (live weight divided by 10 × fat production in 24 hr.), carotene content of the milk fat, stage of lactation, gestation, quantity of food, illness of the cow, nature of the food.

The influence of different factors was calculated by statistical methods, with their internal correlations and partial correlation, and regression coefficients. Thus the differences in refraction of the milk fat existing at a given moment between different cows in the same pasture, largely could be explained and also the course of the average refraction as the grazing period advanced.

The main factors for healthy cows receiving no extra food were age of the cow, fat percentage of the milk and ponderosity, while the carotene content of the milk fat was only an important factor in the beginning of the grazing period and the influence of the lactation stage was merely an indirect one.

In the second place, experiments were carried out with groups of cows of several farmers in the same area, all the cows being on pasture without extra fodder. Beyond the mentioned factors, from these experiments there appeared two other important factors, the soil and the degree of poorness of the pasture.

In the third place, experiments also were made with groups of cows in different provinces of the Netherlands, all the cows being on pasture with-

out extra fodder. It then appeared that apart from ponderosity of the cow and fat percentage of the milk, four other factors, all relating to the composition of the grass, were of importance, namely: percentage of crude protein, percentage of starch-like substances, calcium percentage and chlorine percentage. In all these cases the influence of different factors was calculated as in the first experiments, so that the differences in composition of the milk fat largely could be explained on a quantitative basis.

It thus appeared that the differences in re-fraction of the milk fat may be reduced to a number of factors. It has been shown clearly that it is wrong to look only to a single factor; one has to bear in mind all factors that have proved to be important, and the differences are to be ascribed to different factors at different times. Many suggestions for further investigations were given and a new method was worked out to determine the amount of fat in grass in a simple way. The figures now available on the percentage of fat in grass did not give the impression that this factor is as important as often was thought.

A. F. Tamsma

327. Factors affecting the stability of milk fat and fat soluble vitamins. V. N. KRUKOVSKY. *Can. Dairy Ice Cream J.*, 27, 10: 90. Oct., 1948.

See *J. Dairy Sci.*, 31: 961-972. 1948.

328. The Sanders-Sager test. ANONYMOUS, *Milk Plant Monthly*, 38, 2: 36-38. Feb., 1948.

The Sanders-Sager test used for detecting under-pasteurization of dairy products is based on the fact that all raw milk contains an enzyme, phosphatase, that is destroyed by proper pasteurization. The test can be used on all dairy products, detecting the presence of 1 lb. of raw milk in 2,000 lb. of properly pasteurized milk. It is more sensitive in the case of raw cream, due to the increased concentration of the enzyme in the raw product.

This article outlines the complete procedure for conducting this test, including a pictorial reproduction of the prescribed procedure.

J. A. Meiser, Jr.

329. The Babcock fat test of reconstituted milk. G. M. TROUT, J. R. BRUNNER, AND P. S. LUCAS, *Michigan State College, East Lansing. Milk Plant Monthly*, 38, 2: 52-59. Feb., 1949.

Due to the increased sale of dry milk for re-constituting purposes, a comparison of the results obtained when testing reconstituted milk for fat by the Babcock and Mojonnier methods was made. The whole milk powder was reconstituted in distilled water using a Waring food

blender and then stored at 40° F. for 24 hr. prior to testing.

The results obtained by the Babcock test averaged 0.25% below those obtained by the Mojonnier method. The resulting fat columns frequently were dark and contained char bearing a remarkable resemblance to those columns obtained when testing homogenized milk by the Babcock method.

J. A. Meiser, Jr.

330. Problems involved in ashing cottage cheese for calcium and phosphorus determination. H. B. CLEMONS AND E. A. WINKLER, *Univ. of California, Davis. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc.* Pp. 117-118. 1948.

Difficulty was encountered in ashing unsalted cottage cheese curd by the A. O. A. C. method. By adding 5 ml. of a 0.6% Ca acetate solution to 10 g. of curd, a satisfactory white ash was obtained. The authors point out that in addition to facilitating the ashing of the curd, added Ca acetate makes possible a more complete recovery of Ca and P from cottage cheese curd. By ashing a sample of Ca acetate solution, a blank value for added Ca was obtained. It was suggested that ashing at 600° C. instead of 500° C. might give better results on this type of curd.

H. B. Naylor

331. Viscositätsmessungen an hochprozentigem Rahm. (Viscosity measurements of high fat cream.) English summary. W. MOHR AND J. WELLM. *Die Milchwissenschaft*, 3, 7: 181-185. July, 1948.

The viscosity of cream with 60% fat or more rises rapidly in the temperature range of from 40 to 60° C. A 40% cream at 50° C. had a viscosity of from 5 to 5.38 cp., whereas 80% cream at 50° C. had a viscosity of from 3320 to 6720 cp. Viscosity values for other fat percentages and at 40, 50 and 60° C. are given.

The rapid rise in viscosity in high fat cream is due to the structural properties of the cream. In a cream with over 74% fat by volume, the fat globules are deformed, even though they are packed with the minimum of voids. The deformation of fat globules in cream with over 70% fat is to be considered in the formation of butter since it represents a hitherto unknown intermediary step.

I. Peters

332. First annual review of analytical chemistry. *Food.* B. L. OSER, *Food Research Labs., Inc., Long Island City, N. Y. Anal. Chem.*, 21, 2: 216-227. Feb., 1949.

The food section is one of 11 reviews of the applications of analytical developments to different

chemical industries. Food analysis is considered under the following headings: moisture, carbohydrates, proteins and amino acids, vitamins, inorganic elements, decomposition and contamination, disinfectants, preservatives and insecticides. There are 395 references almost entirely to publications of the last 8 yr. B. H. Webb

Also see abs. no. 280, 290.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

333. Centrifuge for the separation of serum from cheese constituents. G. J. STREZYNSKI. (Assigned to DeLaval Separator Co.) U. S. Patent 2,461,129. 6 claims. Feb. 8, 1949. Official Gaz. U. S. Pat. Office, 619, 2: 490. 1949.

Structural details are given of a separator bowl designed specifically for continuously removing whey from coagulated milk products as a step in the manufacture of soft unripened types of cheese. R. Whitaker

334. Method of producing butter by cooling cream of high concentration. H. O. LINDGREN. (Assigned to Aktiebaloget Separator Corp., Sweden.) U. S. Patent 2,461,117. 3 claims. Feb. 8, 1949. Official Gaz. U. S. Pat. Office, 619, 2: 487. 1949.

Cream containing 81 to 82% fat is obtained by passing 25 to 30% cream through a separator. Without coming in contact with air it passes to a mixer which incorporates salt, coloring materials and flavoring and then to a cooling device which effects a phase reversal, converting the cream into butter. R. Whitaker

335. Measurement of holding time of H. T. S. T. pasteurizers. WM. JORDAN AND R. F. HOLLAND. Can. Dairy Ice Cream J., 27, 10: 76. Oct., 1948.

See abs. no. 232, p. A51.

336. Electronic pasteurization. DAVID LEROI. Milk Ind., 29, 6: 56-58. Dec., 1948.

A system was evolved whereby the milk falls freely through a large tube placed between two electrodes. When the electrodes are joined to the alternating circuit, the temperature of the tube becomes intense, and the milk passing through it is raised to a temperature of 205° F. in 0.067 sec. Milk which had passed through the tube had no trace of a cooked flavor. The bacteria count was considerably less than the 1% obtained with ordinary pasteurization and the vitamin and other nutritional losses are no more than for normal pasteurization. The milk is cooled quickly by injecting it into a vacuum

chamber. The initial temperature is reduced to 35° F. in 0.2 sec. The electronic pasteurization of milk is still in the laboratory stage.

H. Pyenson

337. Homogenizer. A. M. KINNEY AND A. N. JERGENS. U. S. Patent 2,452,661. 16 claims. Nov. 2, 1948. Official Gaz. U. S. Pat. Office, 616, 1: 150. 1948.

The novel feature of this homogenizer is the homogenizing valve, which consists of 2 flat coil springs which are compressed and released alternately by a reciprocating rod. The product under pressure enters the center of one spring, flows through the spaces between the coils and then through a passage to the outside of the second spring, thence through the coils of the second spring to the interior from whence it is discharged. The reciprocating rod passes through the second spring and is attached to a cylinder between the two longitudinal aligned springs. The homogenizing action takes place as the product passes through the vibrating springs.

R. Whitaker

338. Planning, construction and maintenance of modern dairy plants. B. A. BOUCHER. Can. Dairy Ice Cream J., 27, 10: 37-38. Oct., 1948.

In planning new plants and the remodeling of older plants, a complete staff of engineers and architects specializing in creamery plant design can be of considerable assistance to the dairy industry. It is important to plan for future expansion and to arrange the plant equipment so that the product is processed in a continuous flow. The author recommends the installation of the lowered sawtooth roof over all processing areas to provide an abundance of natural light and to assist in the control of moisture. The walls should be of tile or smooth cement plaster. The floors should be constructed either of tile for long life or of concrete for shorter life. Acid-resisting compounds for joints and acid-resisting sealer should be used. The floor should be sloped and have adequate drains. Plant maintenance and sanitation should be borne in mind when planning the plant layout. H. Pyenson

339. Milk plant layout. H. L. MITTEN, JR., Ohio State Univ., Columbus. Milk Plant Monthly, 38, 3: 73-74. Mar., 1949.

Factors to be considered in plant layout are: (a) location, (b) type of building, (c) size, (d) arrangement of rooms, (e) location of equipment and (f) building construction.

Certain rules essential to calculating size are as follows: (a) fluid milk plants require 1 to 2 ft.² of floor space per gal. of milk handled daily; (b) refrigerated storage rooms require 1 ft.² for

each 5.25 gal. of milk handled daily; (c) floor space for processing rooms should be determined by the formula $A = a/0.2$, where A = the floor area and a = area occupied by the processing equipment after a proposed operations expansion; (e) bottle-washing rooms should be sufficiently large to accommodate all necessary equipment and still provide bottle storage space equal to that of the refrigerated milk storage room; (f) dry storage space must be 25% of the total floor area; (g) equipment shall be separated by 2 to 3 ft.; (h) ceilings must be at least 12 ft. high. J. A. Meiser, Jr.

340. High efficiency plant. G. R. JOHNSON, Pace Associates, Chicago, Ill. *Milk Dealer*, 38, 5: 38, 72. Feb., 1949.

An efficient, attractive plant combines economical operation with good appearance, both derived from treating processing requirements, architectural design and engineering as one complete problem. Amplified further it would include: (a) functional planning based on analysis of production to determine specific space and equipment needs, (b) building plant and form most ideally suited to exacting requirements of plant operation, equipment layout and site conditions, (c) construction materials selected for specific uses consistent with economy and ease of maintenance, (d) simplicity of design and structure, keeping initial and maintenance costs at a minimum and (e) appearance, which with simple forms dramatizes use and enhances advertising value. C. J. Babcock

341. Important improvements necessary for creameries. C. A. KERR. *Can. Dairy Ice Cream J.*, 27, 8: 50. Aug., 1948.

A few of the more important improvements necessary in creameries are: (a) temperature control, (b) can washing and steaming, (c) dry creameries in the winter months and (d) improvements in creamery surroundings.

H. Pyenson

342. Practical ammonia refrigeration for ice cream plants. CLYDE H. MINSTER, Greenbrier Dairy Products Co., Beckley, W. Va. *Ice Cream Rev.*, 32, 7: 94-102. Feb., 1949.

The operating cost of a refrigeration system in an ice cream plant is influenced primarily by the amount of power required to produce the necessary tons of refrigeration. To obtain maximum capacity from ammonia compressors with the lowest possible power cost, the following suggestions are made: (a) Operate the compressor at the highest possible suction pressure and still maintain the temperature desired. (b) Use an

evaporator of such capacity that the temperature differential between the ammonia temperature and the temperature of the cooling medium will not exceed 5° F. (c) Use suction lines of such size that the velocity of the gas as it travels back to the compressor will not exceed 4,000 ft./min., thereby avoiding wide differences in suction pressure between the evaporator and compressor. (d) Avoid excessive head pressures which in turn will increase the power cost. (e) Use two-stage compression in ice cream plants where there is a variation in the temperatures which must be maintained. In this system, a booster compressor is connected to the low temperature evaporator and the discharge from the booster is in turn piped to an intermediate cooler for removal of super heat. The discharge from the cooler is pumped directly to the suction side of the second stage compressor. The booster compressor designed for low-pressure, high-speed operation can raise the pressure of the ammonia gas from 0 to 35 lb. with a minimum of power. The second stage compressor operating at 35 lb. suction pressure will in turn have its capacity increased tremendously accompanied by a marked reduction in the power cost per ton of refrigeration produced. W. J. Caulfield

343. The bugs in refrigeration and how to cure them. LEROY WILLIAMSON. *Can. Dairy Ice Cream J.*, 27, 11: 78-82. Nov., 1948.

The article discusses the refrigeration system, which includes the evaporating part (low side), the compressor, the condenser-receiver and the refrigeration supply. Other subjects taken up are suction line, frost line, power element of valve, float valves, blowers, coils, brine cooling, ammonia leakage and excessive oil usage. H. Pyenson

344. Mechanical can washing. C. B. Shogren, Klenzade Products, Inc., Beloit, Wis. *Milk Plant Monthly*, 38, 3: 76-77. Mar., 1949.

Rules that should be observed in operating a straightline can washer are: (a) keep washer free of lime and milkstone encrustations; (b) keep washer in proper mechanical condition; (c) provide at least 30 to 40 lb. of water pressure in the pre-rinse section; (d) recharge the washer with the proper concentration of washing compound daily; (e) maintain overflow between 0.5 and 1 pt./can; (f) insure that sterile rinse pipes and jets are clean and free of lime deposits.

J. A. Meiser, Jr.

345. A study of milk can washing. P. H. TRACY. *Can. Dairy Ice Cream J.*, 27, 9: 86-88, 94. Sept., 1948.

The physical condition of the milk can is not particularly significant bacteriologically except when open seams are present. Washing solutions hot enough (160° F.) to kill the bacteria are important factors in successful can washing. Washing compounds that can withstand high temperatures must be used. Even in a dry can there is apparently enough moisture to support bacterial growth. Washed cans should be kept uncovered and in a dry atmosphere during storage.

H. Pyenson

346. Packaged automatic boilers. WILLIAM PALM. *Can. Dairy Ice Cream J.*, 27, 8: 52-54, 78. Aug., 1948.

By the use of oil or gas fuels it has become possible to arrange for full automatic control of the steam boiler plant. The packaged steam boiler generator was built as one complete unit with boiler, burner and automatic controls all fully assembled, insulated, jacketed, wired, adjusted and tested at the factory. The package boiler is built to operate independently of stack draft. A package type boiler will start cold and be at full operating pressure in 30 min.

H. Pyenson

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

347. Efficiency in plant operation. E. J. FER-
OUSON. *Can. Dairy Ice Cream J.*, 27, 9: 74-76.
Sept., 1948.

Efficiency in dairy plant operation suggests: (a) careful selection of plant personnel, (b) a program of job instruction and training, (c) systematizing of plant maintenance work, (d) a well-planned and -scheduled quality control program, (e) a thoroughly scheduled and detailed program of plant clean-up work, (f) proper scheduling of all work in plant operations, (g) careful standardization of product and (h) elimination of wasteful practices.

H. Pyenson

348. Price and profit. FRED MERISH. *Milk Plant Monthly*, 38, 2: 66-68. Feb., 1949.

Transition from a sellers' to a buyers' market may result in materially lowering a plant's margin on sales. To prevent operating at a loss, plant managers must increase sales and up volume or rely on decreases in the cost of goods sold to counterbalance the price drop.

J. A. Meiser, Jr.

349. Potentialities in efficiency in cheese factory operation. D. M. IRVINE. *Can. Dairy Ice Cream J.*, 27, 9: 78-84. Sept., 1948.

Increased efficiency in cheese factory operation is needed. The commercially profitable manufacture of whey might justify the paying for milk on a total solids basis. If the cheese makers are to obtain an adequate supply of the best quality milk, they must expect to meet competitive prices. Labor constitutes an important item of processing costs, consequently the output per individual must be greater. The more arduous features of the cheesemaking operations must be reduced or eliminated. More cheese was produced per man 40 yr. ago than today. Efficiency can be increased by good roads, improved hauling equipment and mechanized vehicles. In this way the manufacture of cheese can be concentrated in fewer plants. The by-product whey can be utilized better in larger plants. The equipment that will help to increase efficiency is the high temperature short time pasteurizer for cheese-making. Cheese presses are fairly unsanitary and lack temperature control. Standardization of packaging will help to increase efficiency in cheese factory operation.

H. Pyenson

350. Labor's responsibility in the future development of the dairy industry. AUGUST BURNER, Dairy Employees Union, Chicago, Ill. *Milk Dealer*, 38, 4: 44, 86-87. Jan., 1949.

The importance of the different groups to the dairy industry from the employer's standpoint is given as follows: (1) The customer—without consumers, no jobs for anyone; (2) the stockholder—no invested capital, no business; (3) the farmer, production of milk before labor; (4) the employees of the dairy industry. The author agrees that the consumer is of first importance but believes that labor should have priority over the other groups. The following program then is set forth from the labor standpoint: (1) Wages increased in line with current living costs; (2) establishment of sound retirement or pension plans; (3) separation pay to cushion the shock of unexpected lay-off; (4) the working out, on a mutually cooperative basis, of some means of preventing speed-ups and increases in the work load which are harmful to the health of the worker and which reduce the wage-earning span of his life.

C. J. Babcock

351. Bonus plan spurs routemen's sales efforts. ROSS YOUNG. *Milk Plant Monthly*, 38, 2: 76-77. Feb., 1949.

To foster sales to old customers as well as new, the following plan was adopted: Using the previous month's sales records for the base period, routemen received \$1.00 for increased sales totaling up to 5 points, \$1.25 for 6 to 10 points and \$1.75 for all points over 11.

In an effort to insure routemen returning every

possible bottle, a weekly bonus of \$5.00 was given to the man who returned the most bottles in relation to those he delivered. This plan accounted for a 30% increase in bottle returns.

J. A. Meiser, Jr.

352. Annual bonus system builds sales all year. ROSS YOUNG. *Milk Plant Monthly*, 38, 2: 54-55. Feb., 1949.

An incentive sales plan whereby routemen received \$1.50 for every quart over a minimum of 400 qt. increase for the year and 50 cents per qt. for sales over 800 qt. has done much to promote increased sales. This bonus is supplemented by three cash awards of \$1,500, \$1,000 and \$500 for the top three men at the end of the year.

Educational displays showing negligence such as worn-out gears, tires and engines that were damaged beyond repair by improper care were very effective in improving driving habits of the routemen.

J. A. Meiser, Jr.

353. Training milk salesmen. ROSS SIDNEY. *Can. Dairy Ice Cream J.*, 28, 1: 30, 74. Jan., 1949.

The basis of sales training policy should be to impress on the men that conditions prevailing during the war no longer exist. Good dependable service as the customer wants it should be given. The average customer wants: (a) regular and punctual service, (b) accuracy and honesty, (c) a pleasant route salesman with a clean and good appearance, (d) a salesman who knows something about the products he sells and (e) an intelligent answer to questions. H. Pyenson

354. Adding 600 new customers in four months. ROSS YOUNG. *Milk Plant Monthly*, 38, 3: 80-81. Mar., 1949.

For increases of 8 to 15, 24 to 31 and over 32 new customers per month, milk routemen receive a bonus of 50 cents, \$1.50 and \$2.00, respectively. At the end of a 2-mo. period an additional bonus of 25 cents, 75 cents and \$1.00 is paid for all new customers totaling up to 16, 32 and 48. Also, prizes of \$15, \$10 and \$5 are awarded to the three highest-scoring routemen at the end of the first 2-mo. period.

J. A. Meiser, Jr.

355. Retail milk delivery—three days per week. J. M. LAWRENCE. *Can. Dairy Ice Cream J.*, 28, 1: 60, 64. Jan., 1949.

The advantages of 3-day over E.O.D. delivery are a 6-d. plant operation, no relief men are required for the odd day and the housewife gets her milk on the same days of the week. The 3-d.-a-week delivery also improves plant operation by eliminating overproduction, reduces routes by

about one-third, increases employee benefits and increases milk consumption. The disadvantages of 3-d.-a-week delivery are that the consumer has difficulty carrying enough milk over the 3-d. week-end and split accounts are made by the consumer getting milk from two dairies to have delivery 6 d. a week.

H. Pyenson

356. Is your return on investment adequate? A. C. KIECHLIN, Public Accountant. *Ice Cream Rev.*, 32, 7: 44-50. Feb., 1949.

Too much attention has been focused on profit on sales and not enough on the return on invested capital by the ice cream industry. The ultimate yardstick of the profitability of any business enterprise is the return on invested capital, whereas profit on sales is of secondary consideration.

When return on invested capital is low or falls below the return that could be obtained through outside investments it is an indication that something is wrong with the management of the business. Over-capitalization, over-expansion, high credit losses or failure to promote sales effectively are frequent causes of low return on invested capital.

Management should make systematic and periodic checks to determine whether the return on invested capital is adequate. The return on invested capital should be maintained at well above the return that could be obtained on safe outside investments, otherwise there is no point in operating the business. Return on net worth of a business is the most important factor determining the market value of the business and constitutes the true measure of the success of the management.

W. J. Caulfield

357. The truth about profits. L. SPENCER, Cornell Univ., Ithaca, N. Y. *Am. Milk Rev.*, 11, 2: 2-4, 6, 42-44. Feb., 1949.

A study of six milk companies in the New York-New Jersey metropolitan area during the 7 yr. 1941-1947 revealed the following distribution of costs and profits: 57.6% for product, 20.9% for selling and delivery expense, 13.4% for receiving, processing and freight, 4.1% for bottles and supplies and 1.7% for other expenses, leaving 1.0% for profits. The spread between sales and product cost was accounted for as follows: 53.5% for salaries and wages, excluding officers' salaries, 10.3% for bottles and containers, 10.0% for property expense, 9.1% for freight and hauling, 7.0% for other supplies and services, 3.1% for payroll taxes, etc., 2.5% for milk handling charges, 1.0% for officers' salaries and 3.5% for other expenses. During the 7 years notable changes occurred. Dollar sales increased 70%, cost of product increased 88% and expenses of operation increased

45%. Wage rates increased 31 to 75%, with employees in country plants receiving largest increases.

Large companies failed to return a normal rate on investment even in so-called prosperous years. Possible reasons listed were increased store distribution and decreased home delivery, greater difficulty in dealing with labor and political criticism of larger companies. A decrease in spread is possible only if labor efficiency is improved or distribution services are reduced further.

D. J. Hankinson

358. Cost of production in establishing milk prices. C. W. PIERCE. *Can. Dairy Ice Cream J.*, 27, 8: 41-47. Aug., 1948.

Cost of production varies widely among individual farmers. The unsolvable problem arises whether to use the average cost, the cost for the most efficient producers, or a cost which would apply to all but the most inefficient producers. In computing cost per hundred weight it is impossible to determine exactly the cost of the several items that do not represent cash expenditures. It is relatively easy to measure changes in main item production costs from one period to another. Changes in costs, rather than any estimated cost/100 lb. of milk, should be used as one of the guides to the proper level of milk prices. The most inefficient producers should be eliminated from the computation of cost of production.

H. Pyenson

359. Production cost problems in the manufacture of ice cream. H. W. SCHUELKE, Central Dairy Prod. Co., Oklahoma City. *Southern Dairy Products J.*, 45, 1: 33, 42, 43, 46, 47. Jan., 1949.

Since the war the cost of ice cream has increased tremendously and with the resulting advance in prices to the consumer there has come a decrease in volume. The future of the business under these conditions depends more than ever upon the skill of the accountant and the operating manager. All indirect labor costs must be justified by profitable results. The work of all departments must be coordinated so that the product may be delivered to the customer at a profit and at a price at which he will continue to buy in a satisfactory volume. O'Neal Johnson of the International Association of Ice Cream Manufacturers is introducing an efficient cost system for the purpose.

Chief attention should be given to obsolete and inadequate accounting systems, inefficient personnel, excessive distribution systems and unbalanced inventories. Elimination of the practice of supplying the customers with equipment and subsidies would allow a decrease in the price of ice cream to the consumer. Either a reduction

in costs or an increase in volume is essential to future success. The companies that have well-trained men who are alert financially and capable of adjusting to the future will be successful.

F. W. Bennett

360. A producers' incentive plan. MELVIN MAXWELL, Idlewild Dairy, Scottsbluff, Nebr. *Milk Plant Monthly*, 38, 2: 60-61. Feb., 1949.

This plan establishes a bonus year starting December 17 and ending 52 wk. later. Utilizing a base period covering the last 13 wk. of the bonus year, base amounts for each producer are determined by averaging his three lowest weeks of production during the base period. Each producer then is paid a bonus of 50 cents per hundred at the end of the year, in addition to his weekly check, for all milk he produces up to his base amount.

J. A. Meiser, Jr.

361. Your advertising problems. Part IV. W. FRANK WELCH, Pres., The AD-VER-TIS-ER, Inc., Fort Wayne, Ind. *Southern Dairy Products J.*, 45, 2: 66, 67, 74, 75. Feb., 1949.

Outdoor posters are essentially a prestige medium and have a distinctly different function from that of newspaper and radio advertising. The latter are more flexible as to timeliness and are designed to get more immediate sales reaction. Direct mail advertising is highly specialized and may be too expensive except for a brief and specific campaign. It is regarded generally as a supplement to the routine advertising program. Selecting the proper advertising medium requires the matching of the purpose against the advantages of the different advertising media. A combination of several media usually is more effective than only one, provided the budget is adequate for a reasonably full use of more than one medium. If funds are not available for an effective combination of media, the money should be directed into one channel.

The advice of salesmen of the respective kinds of advertising should be helpful. Employment of a reliable agent or agency which is thoroughly acquainted with advertising methods may be advantageous if the budget warrants such an expenditure.

F. W. Bennett

362. The consumer and dairy product prices. BLAKE A. CAMPBELL. *Can. Dairy Ice Cream J.*, 27, 12: 58-62. Dec., 1948.

Prices in the United States advanced faster in 1946 and the first part of 1947 than they did in Canada. During the past year wholesale prices and the cost of living have increased more in Canada than they have in the U. S. Since 1939, personal incomes, adjusted for changes in the cost of living index, have increased 72% in

Canada as compared with 58% in the U. S. A study of retail prices in 20 food commodities in Canada and the U. S. for July, 1948, showed that in most cases prices in the U. S. were higher than in Canada. If Canada had to pay U. S. prices for dairy products, it would cost the average family \$45 a year more for dairy products.

H. Pyenson

363. Press relations and publicity for your dairy. DAN VALENTINE. *Milk Plant Monthly*, 38, 3: 47-48, 50. Mar., 1949.

Press releases must be newsworthy, localized, concise, non-commercial and timely. When written on a printed press release form, they create a sound publicity program for the dairy plant.

J. A. Meiser, Jr.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

364. Preparation and storage of carotene concentrates. H. L. MITCHELL, W. G. SCHRENK, AND H. H. KING, Kansas Agr. Expt. Sta., Manhattan. *Ind. Eng. Chem.*, 41, 3: 570-572. Mar., 1949.

Carotene concentrates are of interest because of the continued shortage and high cost of fish oils. Solid carotene concentrates would be easy to incorporate in rations of farm animals. The effectiveness of several finely ground solids as carriers of carotene were investigated. The less highly refined carriers resulted in more stable concentrates. The loss of carotene after 5 mo. storage at 25° C. with different carriers was: soybean and cottonseed meal, 68 and 60%, respectively; casein, ground sorghum grain and sorghum bran, 72-81%; glucose and sorghum starch, 100%. The antioxidant action of cottonseed meal was increased appreciably by the addition of 2% lactic acid.

B. H. Webb

365. Forage crop management for higher yields. Part III. C. M. HARRISON. *Michigan State College, East Lansing. Hoard's Dairyman*, 93, 2: 859. Nov., 1948.

The conclusions drawn from this study were: (a) forage mixtures vary as to productiveness in terms of hay or grazing with livestock; (b) mixtures containing alfalfa are more productive than straight grass or red clover grass mixtures and, likewise, they are superior as green manure when measured in terms of added corn following in the rotation; (c) pasturing any mixture will result in a greater green manure benefit than removing the forage as hay or hay and pasture; (d) pasturing apparently results in the removal of less mineral nutrients from the soil, making it

easier to reestablish a good forage field with less fertilizer than is the case where the forage is removed from the field.

J. B. Frye, Jr.

366. Pasture control. With special reference to the border area. J. H. PRELLER, College of Agriculture, Potchefstroom. *Farming in S. Africa*, 23, 264: 191-199. March, 1948.

The effects of fertilizer and systems of management on yield of South African pastures are described.

F. C. Fountaine

367. Estimation of digestibility of grazed pasture from feces nitrogen. R. J. LANCASTER. *Nature*, 163, 4139: 330. 1949.

Based on pastures located in England, United States, South Africa and New Zealand, data are presented to show that the nutritive value of the herbage can be calculated from the nitrogen content of the feces of sheep grazed on the pasture.

R. Whitaker

368. Grazing without fields. DAVID LeROI. *Milk Ind.*, 29, 2: 48-49. Aug., 1948.

Hydroponics, or the growing of field crops in liquid culture media without soil, now has passed the experimental stage and a technic has been perfected to yield crops which compare more than favorably with normal soil agriculture. It is possible to raise healthy crops without soil. Specially constructed cabinets have been developed for the raising of cattle pasture. By this method, in 11 d. the first sowing has attained a growth of 10 in. An 80-tray cabinet yields 200 lb. of grass daily.

H. Pyenson

Also see abs. no. 309.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

369. The inheritance of red, roan and white coat colour in dairy shorthorn cattle. I. C. JONES, Univ. of Liverpool. *J. Genetics*, 48: 155-163. 1947.

Old theories and exceptions are reviewed and 856 records from a carefully-kept private herd are tabulated by type of matings. The two-gene explanation of Ibsen (1933) is substantiated with respect to homozygous dominant red and incompletely dominant white. The unusually low number of exceptions, i. e., 11, is considered to be due to the great care of the herdsman. Only one case of error in diagnosing the color was found in later checking the exceptions with the animal or a photograph. To explain the exceptions that it was possible to check, it was suggested that one of the three animals in each case, i. e., calf, sire or dam, that were phenotypically

red were genotypically roan but so extreme in the series that they could not be distinguished. The various grades of roaning are implied to be due possibly to differences in internal temperatures during prenatal growth. Further explanation of these exceptions was considered unsafe.

L. O. Gilmore

370. The Bucks County quintuplets. ROBERT COOK. J. Heredity, 39: 347-348. 1948.

A report is made on a set of quintuplet heifer calves born in Pennsylvania. On the basis of color marking the set is assumed to be composed of one pair of monozygotic twins and one set of monozygotic triplets, hence resulting from two fertilized eggs.

After referring to the literature on the frequency of occurrence of multiple births of different orders, the frequency for the occurrence of quintuplets is estimated at not less than one in 3-5 million births.

L. O. Gilmore

371. The role of major genes in the evolution of economic characters. R. L. KNIGHT, Empire Cotton Growing Corp. and Sudan Govt. J. Genetics, 48: 370-387. 1948.

It is considered that preadaptation (cf., response to existing selection pressure) is common in economic characters. Such characters involving major differences typically will be found to be controlled by one or a few major genes. Different plants are listed for which economic characters are controlled wholly or in part by major genes. For breeding purposes, attempts should be made to reduce complex characters to the action of the individual genes responsible to expedite analysis.

L. O. Gilmore

372. Corynebacterium pyogenes in bull semen. J. R. HANCOCK AND W. R. KELLEY. Vet. Record, 60, 51: 669-670. Feb., 1949.

All samples of bull semen received in the authors' laboratory from December, 1945, to March, 1947, were routinely examined for the presence of *C. pyogenes*. Semen samples were submitted for examination because of breeding difficulties in the herds in which the bulls were maintained, and were collected by the artificial vagina method with precautions to prevent contamination. A total of 170 semen samples from 70 bulls in 40 different herds was studied and *C. pyogenes* was isolated from 25 bulls in 16 of the herds studied. The organism could not be recovered from sheath swabs in known carrier bulls, which indicates it is in the genital tract above the sheath. No difference was detectable between positive and negative bulls as regards semen examination, and in 3 herds with both

positive and negative bulls, no difference was noted in the conception rate. However, herds in which positive bulls were used had a high incidence of genital infection in females, and cultural examinations of vaginal discharges showed *C. pyogenes* present in most cases. The authors suggest that bulls known to produce semen with this organism in it be withheld from service.

R. P. Niedermeier

Also see abs. no. 274.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

373. Painless dehorning. F. S. BARLOW. Hoard's Dairyman, 94, 3: 105. Feb. 10, 1949.

A local anesthetic (not named) is injected halfway between the eyes and the horns 20 min. before dehorning. The head is disinfected at the base of one horn and drawn to one side by a nose lead for the dehorning operation. Practically no pain is experienced by this method. This method is being used by Dr. Harold E. Amstutz, College of Veterinary Medicine, Ohio State Univ.

J. B. Frye, Jr.

374. Suspension hanger. F. W. STANKE AND L. F. BENDER. (Assigned to Universal Milking Machine Co.). U. S. Patent 2,460,856. 4 claims. Feb. 8, 1949. Official Gaz. U. S. Pat. Office, 619, 2: 422. 1949.

A band, passing over the cow's back, supports the milk receiver of a milking machine in such a manner that it is permitted to swing beneath the cow's stomach. The swinging movement of the milk receiver, caused by the pulsations of the flexible milk tubes attached to the teat cups, imparts a tugging action alternately to different portions of the teats.

R. Whitaker

375. Reinforce farm manure. C. J. CHAPMAN, Univ. of Wisconsin, Madison. Hoard's Dairyman, 94, 2: 205. Jan., 1949.

Spreading of superphosphate in loose run barns, sheds or box stalls at intervals of every 4 or 5 d. or weekly at the rate of 1 lb./animal/d. is recommended. Reinforcing animal manures with superphosphate not only increases the value and the effectiveness of the manure by actually bringing up the phosphate content but also helps to prevent losses of valuable nitrogen which may get away in the form of volatile ammonia.

J. B. Frye, Jr.

ICE CREAM

C. D. DAHLE, SECTION EDITOR

376. Ice cream stabilizers. P. H. TRACY. Can. Dairy Ice Cream J., 27, 10: 34-36. Oct., 1948.

A number of satisfactory ice cream stabilizers have been developed within recent years for use under various conditions. The more important ones are gelatin, dariloid, Irish moss, locust bean, sodium carboxymethyl cellulose (C. M. C.), ground psyllium seed husks, karaya gum, oat gum, pectin, quince seed and mechanical mixtures of two or more of the above products together with corn sugar as a carrier. Whipping aids or emulsifiers in conjunction with regular ice cream stabilizers help whipping and air incorporation and give the ice cream a drier appearance.

H. Pynson

377. Nature and properties of some new ice cream emulsifiers. J. S. GOULD AND N. P. TARASSUK, Univ. of California, Davis. Proc. 29th Ann. Meeting, Western Div., Am. Dairy Sci. Assoc. Pp. 119-121. 1948.

Using the emulsifier "Tween 60" and the stabilizer "SherVel," it was found that 0.10% of emulsifier was optimum in mixes frozen in batch freezers, but that 0.15 to 0.20% was required to obtain improved body and texture when a continuous freezer was used. The emulsifier had no effect on the susceptibility of ice cream to heat shock. It tended to slow the melt-down and in some instances caused a curdy appearing melt-down. A given emulsifier may not give identical results with all stabilizers.

H. B. Naylor

378. Consumers preference tests for imitation and pure flavors in ice cream. P. S. LUCAS. Can. Dairy Ice Cream J., 27, 11: 92-94. Nov., 1948.

See abs. no. 119, p. A26.

379. An analysis of concentrated citrus oils. DAVID E. LAKRITZ, Florasynth Laboratories, Inc. Ice Cream Field, 53, 2: 32. Feb., 1949.

The author classifies concentrated citrus oils as follows: (a) "Concentrated" citrus oils—oils from which a portion of the terpenes has been removed. (b) Terpeneless citrus oils—oils from which the major portion of terpenes has been removed. (c) Sesquiterpeneless citrus oils—oils from which the major portions of both terpenes and sesquiterpenes have been removed.

It is pointed out that most essential oils are composed of (a) mixtures of hydrocarbons, principally terpenes, sesquiterpenes and polyterpenes, (b) oxygenated compounds including acids, alcohols, esters, lactones, aldehydes, ketones, phenols and ethers and (c) non-volatile material consisting chiefly of waxes and resins.

The author briefly describes the four principal methods of concentrating citrus oils, namely, (a) vacuum distillation, (b) steam distillation,

(c) alcohol distillation and (d) extraction.

The removal of terpenes (unsaturated hydrocarbons) increases the stability of the remaining oil. It is claimed that such oils are especially useful for flavoring gelatin dessert and are recommended for use in ices and sherbets.

Although the oxygenated components of citrus oils comprise the major portion of their flavoring, terpenes contribute towards flavor and sesquiterpenes and waxes have some fixative action. It is, therefore, recommended that where stability and solubility of oils are not too important that less concentrated and even unconcentrated oils be used.

W. C. Cole

380. Flavoring materials used in ice cream. CARL KOERVER, Pioneer Ice Cream Company (The Borden Co.), Brooklyn, N. Y. Am. Milk Rev., 11, 1: 36-40, 48. Jan., 1949; Can. Dairy Ice Cream J., 27, 10: 78-88. Oct., 1948.

See abs. no. 39, p. A7.

381. Diced cream. ANONYMOUS. Ice Cream Rev., 32, 7: 39, 87. Feb., 1949.

See abs. no. 254, p. A54.

382. Sealtest presents — ice cream eclairs. ANONYMOUS. Ice Cream Trade J., 45, 2: 46. Feb., 1949.

The eclair is an oblong-shaped bar about 5 in. long, consisting of a layer of cake, a layer of fudge and a layer of ice cream, covered with a chocolate coating and 4 dabs of whipped cream. Special equipment makes automatic mass production possible. Dealers pay 45 cents for 4 bars which retail for 60 cents, providing a gross margin of 25% of the selling price.

W. H. Martin

383. The trend in ice cream packages. C. H. SCHAEFFER. Can. Dairy Ice Cream J., 27, 8: 65-66, 70. Aug., 1948.

Package ice cream should not be looked upon as inevitably replacing bulk, but rather for its value in adding to the total sales. One ice cream item should not be pushed to the exclusion of others. Ice cream should be sold in packages most acceptable to the public; package attractively, price properly and let the public be the judge of what they wish to buy.

H. Pynson

383a. Bulk ice cream container. H. B. TILLERY. U. S. Patents 2,459,727 and 2,459,728. 2 claims. Jan. 18, 1949. Official Gaz. U. S. Pat. Office, 618, 3: 942. 1949.

Details are given regarding the construction of a carton for ice cream which may be shipped flat, readily assembled to provide a box having a piston-like bottom that can be pressed out to

eject the contents. The assembled carton is so constructed that air may circulate freely beneath the bottom. R. Whitaker

384. 1948 gallonage. ANONYMOUS. *Ice Cream Trade J.*, 45, 2: 44. Feb., 1949.

Ice cream production in the United States declined 10% in 1948. U.S.D.A. Bureau of Agricultural Economics estimates indicate a production of 568,735,000 gal., which is still 13% more than the 1942-1946 av. and 88% ahead of the av. for 1939, 1940 and 1941. Leading states were Pa., N. Y., Calif., Ill. and Ohio. After experiencing monthly drops in production since March, the production trend turned up in November and December. W. H. Martin

385. Your ice cream cabinet—its relation to your sales and profits. A. C. DOAK, Frigidaire Div., General Motors Corp. *Southern Dairy Products J.*, 45, 2: 26, 28, 41. Feb., 1949.

The location of the cabinet is important in increasing sales. Usually the most effective location is in line of store traffic, near the cash register or used as a dividing counter. Location near bakery goods, the use of attractive advertising and the display of a full line of flavors also are suggested. A neat, orderly arrangement of packages plainly marked to facilitate rapid and courteous service increases the rate of turn-over. An extremely clean cabinet, inside and out, will stimulate buying. Frequent refinishing or polishing is recommended.

High operational and service costs can reduce profits greatly. The use of manufacturer's instructions is helpful in this respect. Frost should be removed when it equals the thickness of a pencil. Allow ample room around the compressor for air circulation. Avoid battering the cabinet. Lubricate regularly as needed. Inspection by a service man every 3 mo. is economical. In case of failure of the compressor to function, examine it for easily-corrected causes of trouble but call the service man when corrective measures are not understood. F. W. Bennett

386. Things to consider when manufacturing ice cream of excellent quality. G. H. WILSTER. *Can. Dairy Ice Cream J.*, 27, 9: 38-42. Sept., 1948.

Requirements for the manufacture of ice cream of excellent quality include (a) a sanitary plant, (b) dairy products low in bacterial content, (c) fruit, flavors, sweeteners, stabilizer, egg and colors of fine quality, (d) modern, clean, well-kept equipment, (e) correct mix standardization, mixing homogenization and freezing, (f) efficient hardening and (g) maintenance of fresh stocks of ice cream. H. Pyenson

387. The pursuit of quality in the manufacture of ice cream. C. W. ENGLAND, High's Dairy Products Co., Washington, D. C., and Baltimore, Md. *Southern Dairy Products J.*, 45, 2: 102-104, 106, 110-113. Feb., 1949.

Some of the essentials for quality are good raw materials, clean and sterile equipment, proper balance of ingredients, equipment in good operating condition, careful processing, quick hardening and uniformly low storage temperatures. An educational program for the employees who supervise or do the actual work will promote quality. Demonstrations especially increase the understanding of the employees.

Plant sampling of the materials and products during the manufacture may eliminate many defective lots. The sampling also should extend to the retail cabinets of the dealers, including comparisons with competitive brands during which identifications of the samples are removed. A complete laboratory analysis of each sample often reveals some valuable facts.

Uniformity as well as level of quality is important. This involves the proper standards and uniformity in titratable acidity, kinds and amount of stabilizer, complete solution of the stabilizer, kind and amount of sugar, the grade of flavoring, dumping and use of returns, control of viscosity, exact weighing of ingredients, checking and standardizing the finished mix, maximum pasteurization temperature with cooked flavor, homogenization which produces small unclumped fat globules and mix free from curdling, fast freezing, over-run control, prompt hardening, proper cleaning and sterilizing procedure for equipment, good housekeeping and careful handling until the ice cream reaches the consumer. F. W. Bennett

Also see abs. no. 292, 316, 342, 356, 359, 389, 411.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

388. Flavors in milk. J. A. NEWLANDER, Univ. of Vermont and State Agricultural College, Burlington. *Milk Plant Monthly*, 38, 3: 42, 44-45. Mar., 1949.

The five common off-flavors described are feed, oxidized, high acid, rancid and cooked. Causes and prevention of these defects are discussed. J. A. Meiser, Jr.

389. Low temperature storage of dairy products. W. S. ARBUCKLE, North Carolina State College, Raleigh. *Quick Frozen Foods*, 11, 7: 92-93. Feb., 1949.

Answers to questions concerning proper freezer storage temperatures for milk and milk products arising in connection with home freezer storage

cabinets and frozen foods locker plants are given. Dairy products must be protected against flavor deterioration and impairment of body and texture. Retail packages of butter and ice cream should be wrapped with protective materials such as plioform or aluminum foil of freezing weight. Butter so protected will keep well for long periods at 0° F. Ice cream likewise may be kept 6 weeks or longer. Holding ice cream at constant temperature is essential to avoid detrimental effects on body and texture. Cream containing 40% butterfat will hold satisfactorily for 3.5 mo. If 10% sugar is added before freezing, there will be less tendency to oil off when the cream is thawed. Whipped cream has not been frozen successfully. Milk, either whole or skim, concentrated or unconcentrated, requires a lower temperature (-15° F.) for storage than home freezer cabinets or lockers maintain. While cottage cheese can be frozen, the result is not satisfactory.

L. M. Dorsey

390. An efficient electric milk pasteurizer for home use. A. V. MOORE, Texas Agr. Expt. Sta., College Station. *Am. Milk Rev.*, 11, 1: 54-55. Jan., 1949.

Tests for bacteria, phosphatase activity, coliform bacteria, flavor and cream volume were made on milk pasteurized in the "Safgard" home pasteurizer. Tests made on five trials indicated satisfactory reduction in bacteria numbers and complete freedom from coliform bacteria. Phosphatase tests were negative in all trials. Heated flavor was not reported in any of the pasteurized samples. Cream volume apparently was not affected adversely, since data show increases in cream volume following pasteurization in some trials and decreases in other trials.

D. J. Hankinson

391. Fat-free vitamin-fortified milk. K. G. WECKEL, Univ. of Wisconsin, Madison. *Milk Dealer*, 38, 4: 47-48, 80-84. Jan., 1949.

The following reasons are given that fat-free milk should be processed for retail sales: (1) Many consumers believe they cannot afford to pay prevailing prices for bottled whole milk; (2) there is a probability that fat-free milk at a lower price may be sold when whole milk cannot; (3) many people are not consuming any whole milk or are consuming it in quantities far less than desirable; (4) many people are evidently conscious of the very good nutritional value of fat-free milk.

The following groups especially would be interested in this product: (1) individuals who are overweight, (2) individuals in whom fat digestion is carried out poorly or incompletely because of enzyme deficiency, (3) individuals in the upper age group when it is necessary to consume less of

the rich foods that tax digestion facilities and (4) individuals on restricted diets because of health conditions.

The article is summarized as follows: "There is a market for fat-free milk. This market is above and beyond that now being ministered by the dairy industry. The product is easily processed, and on the basis of experience, is quite acceptable to consumers. The nutritional quality can be enhanced to equal that of milk, excluding its butterfat, by the simple expedient of adding vitamins A and D. The vitamin-fortified milk has been approved by the American Medical Milk Commission and is being introduced in a significant number of dairies throughout the country."

C. J. Babcock

392. Promoting non-fat fortified milk. ANONYMOUS. *Milk Dealer*, 38, 5: 42, 78. Feb., 1949.

The Supplee-Wills-Jones Milk Co. of Philadelphia, Pa., has been marketing Sealtest Fortified Fat-free Milk since August and they are pleased with the reaction to the product on the part of the medical profession and consumers. Indications are that it has not been replacing sales of whole milk, as about 95% of the sales seem to be additional sales. The product contains less than 0.1% butterfat. It is pasteurized at 185° F. and fortified with 2,000 units of vitamin A and 400 units of vitamin D. The product is marketed at 4 cents under regular market milk, or 1 cent above the previous price for regular skim milk, and reaction to the price has been excellent.

Abbotts Dairies in Philadelphia introduced a non-fat soft curd milk with added vitamins A and D last July. Each quart contains 2,000 units of vitamin A and 400 units of vitamin D. They have applied for patent protection on the soft-curd feature, as their process is new in the field of skim milk. The product is marketed at 1 cent a quart less than standard or so-called "B" milk and 5 cents a quart less than homogenized "A" milk.

A fat-free fortified certified milk is being distributed in a midwestern city. It sells at the same price as regular pasteurized milk. This distributor believes that it would be a mistake to sell fat-free milk at a considerably lower price than regular milk. This belief is based on the fact that in those markets where buttermilk is sold cheap, it is kicked all over the map.

Each of the above distributors has promoted the sale of fat-free milk with the backing of the medical profession.

C. J. Babcock

393. Uniform inspection of milk supply. L. T. TOMPKINS. *Can. Dairy Ice Cream J.*, 27, 8: 68-70. Aug., 1948.

The score card used by the Mass. Milk Regulation Board is divided into five parts: (a) "A"

or *excellent* classification, (b) "B" or *good* classification, (c) "C" or *fair* classification; (d) "D" or *poor* classification and (e) "F" or *unsatisfactory* classification. The card is graduated to five classifications for each of the headings to be classified. Such a score card requires a lot of experience, close coordination and careful study of each unit scored. H. Pyenson

394. Studies on the bacteriological flora and keeping quality of pasteurized liquid cream. E. L. CROSSLEY, April and Barrett Ltd., Yeovil, Somerset. J. Dairy Research, 15, 3: 261-276. May, 1948.

The special conditions of the cream trade in Britain are discussed. Bacteriological control of cream processing was studied over a period of 10 yr. at a large country depot engaged in cream distribution on a nation-wide basis. Sources of bacterial contamination and means of reducing infection to a minimum are discussed in detail.

A 24-hr. grading test as a means of forecasting the probable keeping quality of pasteurized cream was evolved. The test consisted essentially of inoculating 10 ml. of sterilized milk containing 0.01% bromocresol purple with 1 ml. of cream, incubating the tubes at $30 \pm 1^\circ$ C. and examining them after 16-17 hr. and again at 24-25 hr. to grade 4 in which case either definite acid or acid and clot were observable after 16 hr. and both acid and clot after 24 hr.

Data from 2558 samples of heat-treated cream are presented to show the relationship between the grading test, coliform test, colony count and keeping quality. A decline from grade 1 to grade 4 was accompanied by a pronounced reduction in the mean keeping quality, amounting to a difference of roughly 10 hr. between each grade. In general, there was a greater correlation between keeping quality and the coliform test than with mean colony counts at 37° C.

E. L. Thomas

395. How to produce and deliver high-quality cream. E. M. BARKER, Rochester Dairy Cooperative. Am. Milk Rev., 11, 2: 21, 22, 24, 62. Feb., 1949.

Cream of uniform high quality may be produced if (a) milk is graded closely at the intake for flavor and odor, (b) effective sediment-control is maintained, (c) bacteria counts are made of producers' milk with arrangements for follow-up of unsatisfactory milk, (d) clean cans are returned to the farmer, (e) milk is cooled to 40° F. when received if it is to be stored before separating (f) air incorporation is minimized, (g) copper-free equipment is used, (h) equipment is cleaned and sterilized properly, (i) proper pumps are used, (j) equipment of sani-

tary design is used, (k) clean, sterile cans are used for storage and shipment and (l) cans are scheduled and iced properly. D. J. Hankinson

396. Fat variations in milk. Part one—Farm factors. I. A. GOULD AND R. E. SROUT, Univ. of Maryland, College Park. Milk Plant Monthly, 38, 2: 32-35, 44-45. Feb., 1949.

Despite continued efforts to provide harmonious producer-plant relationships, misunderstanding as to the cause of fat variations in milk frequently arises. Basically the causes of fat variations may be divided into: (a) farm factors and (b) dairy plant factors.

The daily weights and corresponding fat tests of milk received at dairy plants do vary because of (a) breed and individual variations, (b) stage of lactation, (c) season of the year and temperature, (d) interval between milking, (e) exercise, (f) herd management and (g) such miscellaneous factors as disease, age of cow and oestrus period.

The comparison of D. H. I. A. tests with plant tests also has accounted for much misunderstanding. However, it must be remembered that there are numerous reasons why the D. H. I. A. tests do not agree with plant tests: (a) plant tests usually are composite tests or periodic spot tests whereas the D. H. I. A. tests cover only a 1-day period (b) usually the manner of milking is changed considerably the day the D. H. I. A. tests are conducted and (c) all the milk tested on the farm actually may not reach the plant due to home usage, spillage, feeding, etc.

J. A. Meiser, Jr.

397. Fat variations in milk. Part two. Dairy plant factors. I. A. GOULD AND R. E. SROUT, Univ. of Maryland, College Park. Milk Plant Monthly, 38, 3: 36-41. Mar., 1949.

Those dairy plant procedures influencing fat variations in milk are: (a) dumping the milk, (b) weigh tank sampling, (c) care and preparation of samples and (d) testing. Proper design and agitation have done much to eliminate errors due to weightank sampling. Where fresh samples serve as a basis of payment, at least five samples should be taken per month, including at least one Sunday or holiday sample. Time-composite samples must be taken proportionately using the proper concentration of an approved germicidal agent, placed in closed containers and stored in a dark room at 45° F. for a period of time not exceeding 2 wk. Testing must be done according to the recommended procedures for the Babcock test.

J. A. Meiser, Jr.

Also see abs. no. 273, 310, 311, 312, 313, 314, 328, 329, 336, 337, 339, 340, 344, 345, 348, 351, 352, 353, 354, 355, 357, 358, 360, 403.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

398. Acetate as a possible precursor of ruminant milk fat, particularly the short chain fatty acids. S. J. FOLLEY AND T. H. FRENCH. *Nature*, 163, 4135: 174. 1949.

In vitro experiments with mammary gland slices indicate that the short fatty acids (C_4 - C_{14}) found in the milk of ruminants are synthesized from acetate, rather than formed from carbohydrates, as in the case with non-ruminant animals. Considerable quantities of acetate are formed in the rumen and absorbed into the blood.

R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

399. Consideration in the utilization of solids not fat in dairy industry. H. H. SOMMER. *Can. Dairy Ice Cream J.*, 27, 9: 42-45. Sept., 1948.

The skim milk solids must be valued more so that the milk fat may be valued less and still leave the price of milk at a level that will stimulate production. The dairy industry cannot compete with other food fats if the milk fat has to carry the major part of the cost of producing milk. With all the outlets for skim milk and condensed and dried skim milk products, approximately 20 billion lb. of skim milk had no market in 1946. A market for skim milk must be found for human food uses without resorting to filled products like filled milk, filled cream, filled ice cream and filled cheese and oleomargarine. We should encourage the greater use of cottage cheese, and skim milk powder and the production of high-testing milk as there are less solids not fat in high-testing milk.

H. Pyenson

400. Dairy foods and the adequate diet. H. A. RUEHE, Univ. of Illinois, Urbana. *Milk Plant Monthly*, 38, 2: 36-37. Feb., 1949.

A discussion of the nutritional importance of carbohydrates, fats and proteins in the human diet is presented. Listing vital nutritional information on the various dairy products, he points out the need for consumer education; this would create a better market for dairy products and also foster nutritional welfare work among the consuming public.

J. A. Meiser, Jr.

401. Nutrition research in the dairy industry. ETHEL AUSTIN MARTIN. *Can. Dairy Ice Cream J.*, 27, 8: 33-37, 72. Aug., 1948.

Dairy industry research studies must be planned to yield new constructive nutrition data which furnish a steady stream of fresh, pertinent facts about dairy products and nutrition information of the type needed to correct and clarify misinformation about dairy products. Approximately 50 industry-supported nutrition research studies now are underway or recently completed in 25 universities and colleges. The projects concerning the individual nutrients in dairy products consist of studies on the fats, proteins, minerals, lactose and the vitamins, riboflavin and niacin. Projects dealing with dairy products as whole foods include infant feeding, milk in breads, milk in dental caries prevention, ice cream in the diet, therapeutic diets and dairy products that supplement the diet of children to improve their nutritional well-being.

Nutrition research, to be effective, must be well-supported, well-planned, purposeful, in line with other nutrition research which is conducted and also keyed to the needs of the industry.

H. Pyenson

402. Dietary needs of special age groups. HELEN OLDHAM, Univ. of Chicago, Chicago, Ill. *Milk Dealer*, 38, 5: 45-46, 118-122. Feb., 1949.

Following a discussion of minimal and optimal requirements as well as recommended dietary allowances, the dietary needs of individuals over 50 are discussed. Available data show that individuals in this age group consume from 300 to 500 less calories per d. than the average young adult. However, there is evidence that the protein and Ca requirements of the individual over 50 are at least as great and possibly exceed those of the average young adult. The data are not in full agreement as to the vitamin requirement but indicate that an intake of approx. 2,000 mg. ascorbic acid, 20 to 90 mg. thiamine and 475 mg. niacin per d. is beneficial. Reasons elderly people fail to get adequate amounts of nutrients are discussed; the use of non-fat dry milk solids and fluid skim milk is suggested as at least a partial remedy.

C. J. Babcock

403. Interpreting assay reports on your vitamin D milk. BARBARA L. CARSON. *Ohio Agr. Expt. Station, Wooster, O.* *Milk Dealer*, 38, 5: 41, 114-116. Feb., 1949.

The commonly-used "line test", involving the measurement of the width of the healed area in the bones of the front legs of rachitic rats when such rats have been fed vitamin D, is described in simple terms and its application to assay of vitamin D milk discussed.

C. J. Babcock

404. Ice cream—a nutritious food. A. C. DAHLBERG, Cornell Univ., Ithaca, N. Y. *Ice Cream Rev.*, 32, 7: 34, 76-82. Feb., 1949.

See abs. no. 256, J. Dairy Sci., 31, 7: A99. 1948.

405. The effect of autoclaving with dextrose on the nutritive value of casein. E. E. McINROY, H. K. MURER AND R. THIESSEN, JR., General Foods Corp., Hoboken, N. J. Arch. Biochem., 20, 2: 256-260. Feb., 1949.

When crude casein was mixed with 0.5 g. of water and 1 g. anhydrous dextrose for every g. of protein and autoclaved 2 hr. at 250° F., its growth-promoting qualities were destroyed for weanling albino rats. This treatment produced a rubbery, dark brown material containing no detectable free amino groups. A similar treatment of casein in the absence of dextrose only slightly impaired its nutritional properties. When the moist mixture of casein and dextrose was air-dried at room temperature, no apparent effect on the growth-promoting qualities of the casein was observed; the biological value of this mixture was comparable to that of the untreated casein control.

H. J. Peppler

406. Amino acid and unsaturated fatty acid requirements of Clostridium sporogenes. G. M. SHULL, R. W. THOMA AND W. H. PETERSON. Univ. of Wisconsin, Madison. Arch. Biochem., 20, 2: 227-241. Feb., 1949.

Vaccenic acid was found to be as active as oleic acid in replacing biotin for *Clostridium sporogenes* (ATC 10,000) grown in a chemically-defined medium. A synthetic preparation of *trans*-vaccenic acid produced only 0.25 of the growth stimulation exhibited by synthetic *cis*-vaccenic acid. Natural vaccenic acid is considered to be of the *trans*-type.

H. J. Peppler

407. Microorganisms in the cecal contents of rats fed various carbohydrates and fats. H. NATH, V. R. BARKI, W. B. SARLES AND C. A. FLVEHJEM, Univ. of Wisconsin, Madison. J. Bact., 56, 6: 783-793. Dec., 1948.

Observations were made on the cecal flora of rats fed sucrose, lactose or dextrin, and butterfat or corn oil. In all cases lactic organisms dominated. Lactose induced much higher plate counts than did sucrose or dextrin. Lactose-fed animals also had greater total cecal contents. Lactose tends to maintain a high coliform population as well as large numbers of aciduric bacteria. Dextrin also stimulates coliforms. Counts of all types of organisms were low in the ceca of animals fed sucrose.

No significant differences in numbers of different kinds of bacteria per g. of cecal contents were observed whether butterfat or corn oil

was fed. However, butterfat-fed rats had heavier cecal contents and, therefore, greater total numbers of cecal microorganisms. Coliform organisms are decreased by increasing fat in the diet but lactic organisms are affected little. The ratio of aerobic to anaerobic plate counts was highest on the dextrin diet, lowest with lactose.

D. P. Glick

Also see abs. no. 290.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

408. Reactions to hot atmospheres of Jersey cows in milk. R. F. RIEK AND D. H. K. LEE, University of Queensland, Brisbane, Australia. J. Dairy Research, 15, 3: 219-226. May, 1948.

Four grade Jersey cows were subjected to various combinations of dry-bulb temperatures from 85 to 110° F. and of absolute humidity from 6 to grains of moisture per ft.³ The animals were admitted twice a wk. for 10 wk. to the air conditioned room in which the desired temperatures and humidity had been produced. They remained in the room for 7 hr. or until the rectal temp. reached 107° F. Animals were tied so as to permit them to lie down or stand at will. Water at atmospheric temp. was offered on all occasions 3.5 hr. after admission.

Rectal temp. rose to higher values with less ready establishment of equilibrium the hotter the condition, but exceeded 107° F. only in the hottest atmosphere studied (110° F., absolute humidity 16 gr./ft.³). Respiratory rate was affected similarly. In both cases, humidity had a marked effect as well as temp., an increment of 0.4 gr./ft.³ (approx. 4%) in humidity having the same effect as 1° F. rise in air temp. The highest av. respiratory rate was 200/min. Pulse rate was essentially unaffected by rise in temp. but tended to rise somewhat with humidity. Evaporative loss was increased markedly by temperature, but much less so by humidity. Neither milk nor butterfat production was essentially affected by the exposures. Blood calcium and phosphate levels fell but the erythrocyte count was unchanged. Behavior changes included some licking, panting, salivation, mild agitation, cessation of rumination and refusal of water.

E. L. Thomas

409. Reactions of Jersey calves to hot atmospheres. R. F. RIEK AND D. H. K. LEE, Univ. of Queensland, Brisbane, Australia. J. Dairy Research, 15, 3: 227-232. May, 1948.

Four grade Jersey calves, 8 wk. old, three female and one male, the progeny of cows used in

a similar investigation (abs. no. 408) were subjected to the same combinations of dry-bulb temperature and humidity as the cows. The calves were fed twice a d. with about 0.75 gal. milk each. When the animals were not in the room, lucerne chaff and a concentrate mixture of bran, pollard and maize meal were allowed *ad lib*.

Rectal temperatures rose rapidly to a higher level than was shown by cows under similar conditions but maintained a steady equilibrium thereafter, except under the most severe conditions. Respiratory rate responses resembled those of rectal temperature, the differences from those of cows being even more striking. Humidity had relatively less effect upon the rectal temperature and respiratory rate responses of calves than of cows. Pulse rate and tidal respiratory volumes were relatively unaffected, but minute respiratory volumes rose. Evaporative loss per unit body weight resembled that of cows (markedly increased by temp.) but humidity had less effect on the calves. Behavior changes resembled those of cows but weakness of the hind limbs was observed at rectal temp. about 106° F. Blood calcium, phosphate, sugar and erythrocyte levels were not essentially affected.

Possible explanations for the lower thermal tolerance of calves, as compared with cows, are considered. These include a suggested lower efficiency of sweat glands and a smaller thermal conductance through the superficial tissues in calves.

F. L. Thomas

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

410. Quaternaries vs. chlorine in bacteria control. E. M. FOSTER, Univ. of Wisconsin, Madison. J. Milk Food Technol., 12, 1: 13-18. Jan.-Feb., 1949.

The use of quaternaries in the dairy industry has been advocated because these compounds have advantages over chlorine. The author has suggested a few of these characteristics, such as prolonged bacteriostatic action. The presence of organic matter does not interfere seriously with their action as compared to chlorine, and produces less irritation than chlorine when used as a rinse solution for udders before milking. However, quaternaries are more expensive than chlorine and usually act more slowly. They are less effective germicides in the absence of organic matter and other unfavorable conditions such as hardness of water, pH and temperature, which further restrict their effectiveness.

H. H. Weiser

411. Sanitation in manufacturing and retailing ice cream. A. E. BERRY. Can. Dairy Ice Cream J., 27, 8: 27-30, 48. Aug., 1948.

Sanitation must include cleanliness and safety against disease. The author speaks primarily about those conditions which prevail in the Province of Ontario. The article discusses the following points: (a) origin of contamination; (b) personnel and handling; (c) utensils; (d) sterilization; (e) methods of sterilization; (f) recent regulations; (g) medical examination of personnel; (h) containers; (i) scoops; (j) cones; (k) a sanitary code for the ice cream industry.

H. Pyenson

412. Detergency. J. C. L. RESUGGAN. Milk Ind., 29, 6: 44-48. Dec., 1948 and 29, 7: 39-43. Jan., 1949.

These articles deal with a discussion of detergents, their nature and use, substances found in them and their importance to the dairymen.

H. Pyenson

413. Quaternary ammonium compounds for creamery churns. A. G. LEGGATT. Can. Dairy Ice Cream J., 27, 12: 32-35, 74. Dec., 1948.

The remarkable affinity of wood for this type of sterilizer makes it difficult to recommend strengths of solution to use for satisfactory sanitization. There appears to be a danger of conditioning the microflora of a churn until it becomes preponderantly gram-negative in character and many organisms of this type have been shown to be associated with flavor defects in butter. The effect of continued absorption should be investigated from the standpoint of stickiness and contamination of the cream by the leaching of the compound. Apparently the generally accepted methods used for evaluating the germicidal efficiencies of disinfectants should not be used for these compounds.

H. Pyenson

414. Control of rodents and insects in dairy plants. E. M. SEARLS. Can. Dairy Ice Cream J., 27, 10: 72-73. Oct., 1948.

Rat and mouse trapping should be done systematically. The old style clap-traps have been found most successful to date. Ten rat traps and ten mouse traps are enough for the average-sized plant. Bait the traps with something the rats like but can't get; peanut butter, bacon rind, cheese, smoked fish, raw meat, raw fish, vegetables, bananas and other fruits are all good baits. All traps should be baited and set out the same evening. Rodent-proof barriers must be placed in all the openings.

D.D.T. in the proper form seems to answer all the requirements for a good insecticide. A

5% D.D.T. in odorless kerosene has been found the most satisfactory form for use in a dairy plant as a residual spray. H. Pyenson

protection and quick-starting during cold weather, application of a 0.125 in. coating to valve covers, valve push rod covers and oil pans has been most effective. J. A. Meiser, Jr.

MISCELLANEOUS

415. Undercoating protects truck bodies. TED KNIGHT. Milk Plant Monthly, 38, 2: 74-75. Feb., 1949.

Coating the undercarriage and body interiors of delivery trucks increases the life of the unit by preventing corrosion due to the salt air and lactic acid, and insulates the unit against summer heat. The thickness of the coat applied ranges from 0.125 to 0.1875 in. For added motor

416. Food poisoning. K. R. STEVENS. Can. Dairy Ice Cream J., 27, 8: 102-104. Aug., 1948.

Characteristic symptoms of the various kinds of food poisoning, causes of the poisoning and how they may be prevented are summarized. The food poisonings mentioned are ptomaines, *Salmonella*, staphylococcal, botulism and chemical food poisoning caused by arsenic, lead, cadmium, fluoride, methyl chloride and tin.

H. Pyenson

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

417. **Optical method of testing milk for relative uniformity.** A. A. McMURRAY. U. S. Patent 2,460,101. 2 claims. Jan. 25, 1949. Official Gaz. U. S. Pat. Office, 618, 4: 1141. 1949.

The milk from the various udder quarters of cows is tested rapidly for mastitis by this device, which consists of a dark-colored plastic disc suspended in a horizontal position in a cup. Milk from one quarter is filmed evenly over the surface of the disc. A squirt of milk from another quarter then is directed on the disc; if normal, no change is apparent; if mastitic, a difference immediately is noted in the color of the milk blend on dark disc, due to the dilution caused by the watery character of the mastitic milk. Milk from the remaining two quarters is tested in an identical manner.

R. Whitaker

BUTTER

O. F. HUNZIKER, SECTION EDITOR

418. **Das "Fuer" und "Wider" der verschiedenen Butterungsverfahren.** (The pro and con of different buttermaking procedures.) English summary. M. E. SCHULZ AND W. SCHULZ. Die Milchwissenschaft, 3, 8: 213-224; 3, 9: 253-259. Aug. and Sept., 1948.

The advantages and disadvantages of butter-making by the churn, Fritz and Alfa processes were studied in three plants with daily outputs of butter from (a) 7,000, (b) 10,000 and (c) 50,000 l. of milk. The overhead cost declined in plants *a* and *b* from churn to Fritz to Alfa process, whereas in *c* Alfa was highest with churn and Fritz being similar and lower. The smaller floor space required by the Fritz and Alfa processes was of advantage in *b* and *c*, whereas in *a*, with extra floor space available, this advantage was not considered important. Most time was saved in *a* by the churn process, in *b* by the Fritz and Alfa processes and in *c* by the Fritz process. Most advantageous printing of butter was done in *a* with churn butter, in *b* with churn or Alfa butter

and in *c* with Alfa butter. No preference in method or saving in personnel was reported in *a* and *b* with any of the methods, whereas the Fritz process was preferred in *c*. Plants *b* and *c* found the churn best for churning sour cream or reworking of butter. All plants favored the churn process for sale of fresh buttermilk. The operation costs were lowest for the churn process and highest for the Alfa process. Fat losses were lowest in all plants with the Fritz and Alfa processes. Moisture control was simplest with the churn and most difficult with the Fritz process. The churn process was given preference for suitability to make sour cream butter and for greatest suitability for salting and coloring of butter, as well as for avoiding body defects at all seasons. The Fritz process ranked second in the last item. Incorporation of air into butter was least in the Alfa and highest in the Fritz process. Oiling-off of butter was least in the churn and highest in the Fritz butter. Working of butter without loss of moisture was best with churn and poorest with Fritz butter. The Fritz process permitted best moisture distribution and easiest cleaning, whereas the churn was poorest. The keeping qualities of both Fritz and Alfa butter were similar and much superior to churn butter.

I. Peters.

419. **Process for making butter.** G. W. SHADWICK. (Assigned to Beatrice Creamery Co.) U. S. Patent 2,463,915. 15 claims. Mar. 8, 1949. Official Gaz. U. S. Pat. Office, 620, 2: 581. 1949.

Cream is saturated with an inert gas under pressure and then released to atmospheric pressure to form a mass similar to whipped cream. Phase reversal and the consequent production of butter result when the expanded mass is agitated and worked.

R. Whitaker

420. **Kompletterande provning av kärnäلتare. Medellande nr 167 (ny foljd) fran Statens Maskinprovningar.** (Tests on type W45 churn.) E. SAMUELSSON, Alnarp, Akarp. Mejeritekniska Medd., No. 5-6: 81-83. Dec., 1948

The motor-driven churn, type W45, was found to be very satisfactory when used experimentally for making butter from both sweet and sour

cream. It proved to be especially practical and simple to operate. The observation period was too short for judging the durability of the churn. The churn drum was elevated, thus permitting easy emptying by dropping the butter into a tray placed under the churn.

G. H. Wilster

421. Det kugleformende og frie Fedt i Smør, fremstillet efter Fritz-Metoden, i Forhold til nogle Fremstillingsbetingelser. (The influence of some manufacturing data upon the globular and free fat in butter manufactured by the continuous Fritz-process.) English summary. N. KING. Nord. Mejeri-Tid., 13, 11: 3-6 1947.

The changes in the entrance-temperature of the cream and in the capacity of the Fritz continuous churning machine involve corresponding changes in the percentage of the globular fat and in the number and average volume of the fat globules encountered in the butter manufactured by this method.

The percentage of the globular fat increases at constant temperature with the increasing capacity linearly, as the treatment intensity exerted on the fat globules of the cream diminishes. At constant capacity the percentage of the fat diminishes with the increasing entrance temperature of the cream, as the globules with increasing temperature grow softer and therefore are destroyed more easily. Linear relations also exist between the number and the average volume of the fat globules on one side and the capacity of the churning machine on the other side. The difference in the capacity and the entrance-temperature also influence the form of the fat globules. Lower temperature and higher capacity minimize deformation of the globules. The globules were regularly round and surrounded by a finely-formed bright birefringent edge (consisting probably of oriented tiny fat crystals). With higher entrance-temperature of the cream and with lower capacity, in contrast, the globules were strongly deformed.

G. H. Wilster

422. Konsistenz der Butter. (The body characteristics of butter.) English summary. W. MOHR AND J. WELLM. Die Milchwissenschaft, 3, 8: 232-242. Aug., 1948.

Samples of summer and winter butter made by the churn, Fritz and Alfa processes were examined for body characteristics by the following methods: (a) cutting resistance, (b) cone flow point, and (c) plastometer. All three methods were used with equal success. Since the values obtained by the three methods did not fall on the same point on the flow curve of butter, the results cannot be considered as strictly proportional. By means of the above methods it was possible to detect

invisible differences in the structure of butter, such as crumbliness, oiliness and layer formation. The three methods offer great possibilities in studying the structural characteristics of butter, since each method expresses thixotropic changes of butter in a different manner.

I. Peters

423. Butter slicer. P. H. MORSE. U. S. Patent 2,464,339. 3 claims. Mar. 15, 1949. Official Gaz. U. S. Pat. Office, 620, 3: 840. 1949.

Butter in block form automatically is advanced forward, step by step, as a slicing knife cuts off rectangular shaped portions of butter suitable for wrapping for retail trade.

R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

424. Over de oorzak der gasvorming in korstlose kaas. (On the cause of the gassy fermentation in processed cheese.) English summary. J. W. PETTE AND J. L. LIEBERT, Rijkslandbouwraproefstation, Hoorn, Holland. Verslag. Landbouwk, Onderzoek., 54, 2: 1-23. 1948.

A bacteriological investigation was made of different kinds of gas-fermenting bacteria in blown processed cheese. In hard process cheese (maximum water content, 50-55%) lactate-fermenting butyric acid bacteria (*Clostridium tyrobutyricum*) nearly always caused the blowing; sometimes propionic acid bacteria did so, too. In soft process (maximum water content, 58-63%) the same was true for the butyric acid bacteria; in a few cases putrifying bacteria played a part.

In trying to cause gassy fermentation by inoculating butyric acid bacteria into process cheese, several factors were investigated. If the maximum temperature of processing was below 70° C., blowing occurred, if higher it generally did not occur. Possibly lactic acid bacteria, which can develop after low processing temperatures, cause better anaerobic conditions for the butyric acid bacteria. Processing should be done at so high a temperature that nearly all lactic acid bacteria are killed. Probably lactic acid bacteria only lower the redox-potential. This seems to be very important for the gassy fermentation. High processing temperature may give blowing if the redox-potential is lowered in another way, i.e., by metabolic processes of *Escherichia-Aerobacter* bacteria. This kind of "early blowing" cheese should not be processed together with "late blowing" cheese.

It is not advisable to store at a high temperature a finished product wherein "late blowing" cheese was processed, because this would give a good chance for lactic acid bacteria to develop and thus for butyric acid, also.

A. F. Tamsma

425. De invloed van kaliumnitraat op de boterzuurgisting in kaas. (The influence of potassium nitrate on the butyric acid fermentation in cheese.) English summary. E. A. Vos, Rijkslandbouwproefstation, Hoorn, Holland. The Netherlands Milk Dairy J., 2, 4: 223-245. Oct.-Dec., 1948.

To know more about the influence of KNO_3 on the butyric acid fermentation in cheese, experiments were done with *Clostridium tyrobutyricum* Van Beynum and Pette. In liquid and solid culture media and in Edam cheese the influence of nitrate and nitrite was investigated. In liquid media KNO_3 was reduced, giving per mol. KNO_3 about 3 mol. H_2 less in the fermentation gas. In solid media with 10% gelatin, this reduction mostly did not take place at all, but sometimes was observed to a small extent. In culture media the nitrate could not stop butyric acid fermentation, although nitrite concentrations of 0.007% were effective in liquid media. The nitrite disappeared rather quickly, being unstable in acid organic solution. In cheese nitrate concentrations of 0.03, 0.01, 0.005 and 0.0025% added to the milk stopped the butyric acid fermentation. The spores were not killed but did not develop. This effect was not caused by nitrite, because no more nitrite could be detected in the experimental cheeses than in the control cheeses. Experiments with nitrite used instead of nitrate showed about the same effect in cheese. Although nitrate, and especially nitrite concentrations, decreased rather quickly, they always stopped the development of butyric acid bacteria. This action was ascribed to an increased oxidation-reduction potential in the cheese. The strongly anaerobic butyric acid bacteria cannot develop at the higher potentials, but are not killed. Measurements of the oxidation-reduction potential were carried out, using gold or platinum wires as electrodes. These were driven into the cheese and the hole in the rind of the cheese around the electrode sealed with liquid paraffin. Without nitrate the rH (Clark's rH value) was about 3.7; in case of butyric acid, fermentation values below 1 were found. With nitrate added the figures averaged about 7.5, indicating that this difference might cause the effect. Nitrate and nitrite are supposed to influence the potential via metabolic processes of bacteria. The potential drop in case of butyric acid fermentation is observed some weeks before gas holes develop and before a development of butyric acid bacteria is shown by bacteriological analysis.

A. F. Tamsma

426. Kyllagringsförsök med ost. (Low-temperature storage experiments with cheese.) ANONY-

MOUS. Svenska Mejeritidningen, 40, 44: 408-410. Oct., 1948.

Milk production in Sweden is about 50% higher in June and July than in Oct. and Nov. Experimental work was undertaken to determine the most satisfactory conditions for storage of cheese to maintain high quality economically. The study was made on a total of 47,000 kg. cheese. The results were published by K. E. Thomé, T. Bergman and S. Hoff in "Meddeland nr. 22" from Swedish "mejeriförsök." The cheese varieties were herrgards, svecia and gouda. Half the cheese used in the experiments was stored on shelves in the usual manner and half was placed under refrigeration at a low temperature. All the cheese was judged at 2-mo. intervals while stored for 8 mo. Composition, flavor and aroma were noted carefully and recorded. A better quality cheese resulted from low-temperature storage. It was concluded that storage of cheese under refrigeration was a distinct advantage to those in the cheese industry in Sweden.

G. H. Wilster

427. Mekanisk vändning av osten på lagret. (Mechanical turning of cheese on the shelves.) ANONYMOUS. Svenska Mejeritidningen, 40, 21: 224-225. May, 1948.

For the mechanical turning of cheese, an apparatus developed by G. Jönsson, Malmö Nya Mejeriförening, is used. This consists of two perpendicular side pieces, the same height as the cheese curing room and furnished with wheels or casters. The equipment moves on rails fastened either to the ceiling or laid on the floor. The two side pieces are joined by a center shaft which can be made to move either upward or downward. This center shaft is made with axles and two tongs which can grip the cheese shelves and turn them.

An illustration is given of the numbered shelves and how they are moved and turned mechanically with the cheese upon them. Shelf number 3 with cheese lying flat upon it is moved gently out toward the next row of shelves and then tilted upright, a brace holding the cheese standing on it, as the shelf glides over to shelf no. 16 just across from shelf 3 on the illustration. The cheese now is turned off mechanically onto shelf 16, so the side which was on top now is on the bottom. In like manner all the shelves with the cheese are moved and the cheese turned over mechanically.

Several construction problems remain to be solved before mechanical means for turning cheese can be used widely.

G. H. Wilster

Also see abs. no. 429, 443.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

428. Removing ultimate moisture from powdered products. A. C. BEARDSLEE. (Assigned to the Borden Co.) U. S. Patent 2,465,963. 2 claims. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1539. 1949.

Powdered food products, such as milk, having a low final moisture content, first are dried in the conventional way and then further dried by passing through a chamber maintained under a vacuum not exceeding 20 mm. mercury. Heat is applied to the outside of the chamber and air is admitted to facilitate movement of the product and the evaporation of the moisture. After cooling under reduced pressure, the product with a low moisture content is packed in sealed containers.

R. Whitaker

429. The preparation of dried whey-potato mixtures. ALBERT H. STEVENS, U. S. Dept. of Agr., Washington. Natl. Butter Cheese J., 40, 4: 28-29, 60, 62. Apr., 1949.

A mixture of cheese whey and potatoes, combined in the proper proportions, can be dried successfully. The resulting product is important because of its nutritional value and as a means of utilizing surplus production. The whey-potato mixture can be used in making bread, cakes, wafers, pancakes, doughnuts, dried soups and as an animal feed.

The whey-potato mixture can be dried by either the spray or drum process. The titratable acidity of the whey will influence the amount of potato solids needed to produce a film on the drum drier. For drum-drying, 32.5 lb. of potatoes are used per 100 lb. of cheese whey of acidity not to exceed 0.38%. The potatoes are washed, cooked until soft, ground and combined with preheated (165° F.) whey, agitated for 10 min. and homogenized at 1,500 lb. pressure. Prior to drying, the mixture is preheated to 160° F. It is dried using 50 lb. steam pressure for a maximum drum speed of 12 rpm. or 100 lb. of steam pressure for a maximum drum speed of 20 rpm. The clearance between the rollers should be the same as that used for drying skim milk. H. E. Calbert

430. Manufacture, use and storage of dehydrated, sweetened, condensed skim milk. A. T. MUSSETT AND W. H. MARTIN, Kansas State College, Manhattan. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 15-22 Oct., 1948.

See abs. no. 88, p. A19.

431. The Babcock fat test of reconstituted milk. G. M. TROUT, J. R. BRUNNER AND P. S. LUCAS, Michigan Agricultural Experiment Station, East Lansing. Am. Milk Rev., 11, 3: 48-49. Mar., 1949.

See abs. no. 329, p. A71.

Also see abs. no. 443, 447.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

432. Direct microscopic clump counts of pasteurized milk by carbolated Newman-Lampert no. 2, and the acid- and water-free methylene blue staining procedures. B. S. LEVINE AND L. A. BLACK, U. S. Pub. Health Service, Cincinnati, O. J. Milk and Food Technol., 12, 2: 69-74, 83. Mar.-Apr., 1949.

The effect of laboratory pasteurization of raw milk on direct microscopic stains was studied. Carbolated Newman-Lambert no. 2 and acid- and water-free methylene blue stains were used. Two lots of 25 milk samples were studied microscopically after the samples were held at 4° C. for 24 hr.

Post-pasteurization counts appeared to be the greatest with carbolated methylene blue stain. The pasteurized samples held in the refrigerator for 24 hr. reduced this loss by 12% by logarithmic average and 15% by arithmetic average, using Newman-Lampert stain. The count was less on the pasteurized milk than when using the carbolated stain. Acid- and water-free stain showed a diminution in bacterial counts on post-pasteurized samples and the counts were lowest after storing the samples in the refrigerator for 24 hr. The authors believe there is no evidence to support the opinions that pasteurization of milk causes the bacteria to disintegrate during the cooling period. Heating may cause a denaturation of bacterial proteins and also affect the buffer system of the milk, thus influencing the adsorption of the dye by the bacteria.

H. H. Weiser

433. Om koliprovot. (For bacteria calculations.) A. LEESMENT. Mejeritekniska Medd., no. 2: 17-19, 26-27. Apr.-May, 1948.

A new method for counting bacteria in milk has been tried. Measuring the milk was done with a calibrated platinum loop which contained 0.033 ml. milk. Experiments showed that the platinum loop was just as useful in estimating the bacteria when streaking on media in petri dishes or in Bergman flasks as when estimates were done with roll flasks. Estimation of colon organisms with the roll culture method proved highly successful. The results were less variable than with the streak method. The saving of media was a

noteworthy factor since about 3 ggr. (?) less was needed for a culture by the roll method than by the petri dish method. The roll method therefore would seem to be suitable for laboratories with large numbers of milk samples to be examined. Some special equipment is necessary.

G. H. Wilster

434. Ein Universal-Agarnährboden für das molekerei-bakteriologische Laboratorium. (A universal agar medium for the bacteriological dairy laboratory.) English summary. A. TODOROFF AND K. ASSENOWA. *Die Milchwissenschaft*, 3, 9: 263-266. Sept., 1948.

Whey agar, nutrient agar and wort agar were tried alone or in various combinations with single cultures of *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus helveticus*, *Escherichia coli*, *Aerobacter aerogenes*, *Bacillus subtilis*, a lactose-fermenting yeast, a pellicle-forming yeast and *Penicillium camemberti candidum*. All lactic bacteria tried preferred the combined whey and nutrient agar to the prescribed nutrient agar plus 1% lactose. All test organisms showed good growth and development on a universal medium containing two parts of whey agar, two parts of nutrient agar and one part of wort agar at pH 6.5. This universal medium is recommended to replace the three separate media and the lactose medium formerly used in dairy laboratories in Germany.

I. Peters

435. High colony counts in pasteurized milk caused by bacteria from efficiently sterilized plant. C. S. MORRIS AND M. EDWARDS, Natl. Agr. Advisory Serv., Sarcross, Devon. *Proc. Soc. Applied Bact.*, 1947, 1: 21-23. 1947.

Samples of pasteurized milk from one dairy consistently were found to have colony counts of about 10 million/ml. after storage at 18° C. for 24 hr., although counts before storage were less than 10,000/ml. prior to the incubation. Swabbings from equipment were made, inoculated into pasteurized milk and incubated 24 hr. at 18° C. Pipe lines, the filler bowl, filler valve and bottles harbored the organisms which were capable of the rapid growth at 18° C. These bacteria were present only in small numbers on the equipment. The bacteria were gram-negative and did not ferment sugars or effect any change in litmus milk. They also were not detected by the resazurin test, the test originally recommended for use in conjunction with the storage quality test. M. L. Speck

436. The incidence of thermophilic organisms in farm milk supplies with some observations on the dominant types. S. B. THOMAS, Natl. Agr. Ad-

visory Serv., Crosswood, AND E. J. EVANS AND L. B. JONES, Natl. Milk Testing Serv., Brynawel, Aberystwyth. *Proc. Soc. Applied Bact.*, 1947, 1: 15-18. 1947.

Milk samples, collected from cans on arrival at the milk plant, were stored at atmospheric shade temperature until 22-28 hr. old and then 10 ml. volumes were pasteurized at $63.5 \pm 0.1^\circ$ C. for 30 min. Plates were poured using yeastrel milk agar and were incubated at $30 \pm 0.5^\circ$ C. for 4 d. Thermophilic counts were found to be the highest during Dec.-Feb. and June-Aug. Careless cleaning of equipment was found to be the cause of the winter peak, while higher initial thermophilic counts of summer milk were considered the cause of the peak at that season. Hypochlorite and boiling water sterilization of utensils were found to be very effective in keeping the thermophilic counts low; steam was moderately effective; warm water washing was very ineffective. The types of thermophilic bacteria isolated in their order of occurrence were: microbacteria (approx. 70%), followed by micrococci (and sarcina), spore-formers, gram-negative rods, streptococci, actinomycetes and yeasts. The method of utensil sterilization had no effect on the order of the occurrence and but slight effect on the % of these types of bacteria present in the milk.

M. L. Speck

437. The seasonal incidence of thermophilic organisms in farm milk supplies. S. B. THOMAS, P. M. HOBSON AND P. M. FRANKLIN, Natl. Agr. Advisory Service, Trawscoed, Aberystwyth. *Dairy Ind.*, 14, 1: 31-37, 44. Jan., 1949.

Monthly thermophilic bacterial counts were obtained on milk from 210 farms over a period of 1 yr. The thermophilic bacterial content, both at 30 and at 37° C., of farm milk supplies of poor as well as of fair hygienic quality generally was highest in summer and lowest in winter. This is to be expected, since the incidence of high thermophilic counts increases with an increase in raw milk colony counts.

Milk supplies handled in thoroughly sterilized utensils, so that the heat resistant bacteria rarely exceeded 10,000 per ml., had a higher incidence of thermophilic organisms in winter than in summer. Probably farms with facilities for steam sterilization apply it more carefully and more consistently during warm weather, thus reducing the thermophilic colony counts during summer.

G. H. Watrous, Jr.

438. A modified procedure for determining the thermophilic bacterial content of milk. D. A. MCKENZIE AND J. LAMBERT, Provincial Laboratory, Leeds 6. *Proc. Soc. Applied Bact.*, 1947, 1: 19-20. 1947.

The method is based on that of Myers and

Pence. Samples of milk are pasteurized and then 0.01 ml. of the sample, as obtained by a calibrated loop, is inoculated into yeastrel agar contained in a 2-oz. flat medical bottle. Then, as is customary in the oval tube technic, the bottles are laid on a flat side and the agar allowed to solidify. Incubation is for 3 d. at 30° C. Thermoduric counts made by this method were lower than those made by the plating method. Therefore, it was proposed that the maximum count allowable by the plate method be reduced by 50% in the modified technic. It was suggested that a keeping quality test be performed simultaneously with the thermoduric count, as the latter alone cannot be used to determine poor production methods.

M. L. Speck

439. Preliminary observations on various temperature characteristics of some facultative psychrophilic bacteria. C. V. CHANDRA SEKHAR AND N. WALKER, Univ. College Wales, Aberystwyth. Proc. Soc. Applied Bact., 1947, 1: 24-27. 1947.

Some of the cultures which grew during the refrigeration of milk grew in broth and on agar at 3-5° C. but the optimum temperature was about 24° C. Similar results were obtained with the fermentation of carbohydrates and certain other biochemical tests. CO₂ production was greater at 24 than at 37° C. by two cultures, while a third produced more CO₂ at 37° C. Lactate dehydrogenase activity of six cultures showed the temperature optimum to be at 44° C. M. L. Speck

440. Die Milchsäurestreptokokken und Degenerations-Erscheinungen im Säurewecker. (The lactic acid streptococci and their degeneration in starters.) English summary. A. HADZIANITIC. Die Milchwissenschaft, 3, 9: 260-263. Sept., 1948.

Sixty-five per cent of the strains isolated from a commercial starter were constant in their morphological characteristics, whereas the other 35% were not. Degenerated *Streptococcus cremoris* strains, forming pairs or short chains, were regenerated in size and chain length by from five to seven transfers in litmus milk containing from 1 to 100 mg. potassium meta bisulfite per 100 ml. of medium. A similar treatment with constant *Streptococcus lactis* strains did not result in chain formation. Potassium meta bisulfite and ascorbic acid were used for the regeneration of *S. lactis*.

The step-wise degeneration of starter cultures begins with decrease in aroma, followed by decrease in volatile acids and slow lactic acid production. Mixed cultures are more resistant toward degeneration than are single-strain cultures. The most ideal growth conditions for both the constant and variable strains exist under facultative

anaerobic conditions, such as are in the lower level of the starter. For best results inoculum should be taken from the bottom of the mother culture. I. Peters

441. Coliforms, their significance and control in ice cream making. G. W. SHADWICK, Beatrice Foods Co., Chicago, Ill. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 69-73. Oct., 1948.

The presence of coliform organisms in ice cream does not mean necessarily that the product is unsafe from the public health standpoint or that fecal material is present. However, it may be an indication of careless and unsanitary conditions of manufacture. A list of steps that should be taken in the manufacturing plant in order to eliminate contamination with coliform and other organisms was presented. H. B. Naylor

442. The testing of frozen eggs for pathogens. S. E. HARTSELL, Purdue Univ., Lafayette, Ind. J. Milk and Food Technol., 12, 2: 107-108. Mar.-Apr., 1949.

A comparative study was made on frozen eggs to evaluate the usefulness of different differential culture media in determining the longevity of certain pathogens in the product. *Staphylococcus aureus*, *S. typhosa*, *S. oranienburg*, *S. aertrycke* and *Escherichia coli* were able to survive in frozen eggs up to 10 mo. at -17.8° C. (0° F.). The culture media used were glucose tryptone agar, yeast water and veal infusion agar in addition to selective media—desoxycholate agar, MacConkey agar and *staphylococcus* medium no. 110. The selective media tended to inhibit all of the test organisms, except *S. aureus*, while the nutritive media increased the growth of the pathogens. The author suggested a more satisfactory plating culture medium should be devised for testing the presence of pathogens in frozen eggs. H. H. Weiser

443. Framställning av myölksyra ur vassle. (The preparation of lactic acid from whey.) G. NILSSON, Mjölkscentralens Centrallaboratorium. Svenska Mejeritidningen, 40, 20: 207-210. May, 1948.

It is possible from the results obtained in the experiments to state that under non-aseptic conditions for a period of 48 hr. at 43° C. the milk sugar in whey was changed to lactic acid with a mixed culture of a lactobacillus and a mycoderma, accomplished in such a manner that the fermentation took place with a pH which did not differ particularly from the optimum value (5.6-5.8). The production of Ca lactate in creameries is described. G. H. Wilster

444. Method of enhancing the yield of yeast in a whey medium. A. M. HANSON, N. E. RODGERS AND R. E. MEAD. (Assigned to Western Condensing Co.) U. S. Patent 2,465,870. 1 claim. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1516. 1949.

After adjusting the pH to between 1.5 and 3.5 the whey is heat sterilized, after which the pH is adjusted to between 5 and 8 and then inoculated and cultured. R. Whitaker

Also see abs. no. 424, 425, 481.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

445. Verfahren zur Bestimmung des Vedorbenheitsgrades von Fetten. (Procedure for the determination of degree of fat deterioration.) English summary. H. SCHMALFUSS Die Milchwissenschaft, 3, 8: 225-233. Aug., 1948.

Six approved methods for the detection and determination of spoilage in fat are described and discussed. The methods are those for measuring fat acidity of colored and colorless fats, peroxides, free aldehydes, epihydrinaldehydes and ketones.

I. Peters

446. Methode ter bepaling van de hoeveelheid gekristalliseerd vet in room. (Method for determining the quantity of crystallized fat in cream.) English and German summaries. W. ADRIANI AND A. F. TAMMSA, Laboratorium der Coöperatieve Fabriek Van Melkproducten, Bedum, Holland. Verslag. Landbouwk. Onderzoek., 51, 6G: 79-90. 1945.

The dilatometric method for cream yielded lower values for the amount of crystallized fat than it did for fat from the same cream. As the last value agreed very well with the results of the manufacturing process of butter, the dilatometric results for cream were unreliable. Sources of error are discussed. A new method based on thermic principle was elaborated, giving reliable results for the quantity of crystallized fat in cream. The latent heat necessary to change partly crystallized cream fat into liquid fat was determined, being a good indication for the percentage of crystallized fat. The determination was arranged in a simple way by using as calorimeter a thermos bottle, closed with a rubber stopper and a thermometer accurate to 0.01° C. The cream is mixed in this bottle with hot water and the temperature effect found by extrapolating the curves of temperature decrease before and after the cream was poured into the bottle. Constants necessary for calculating the results can be found easily with the same kind of experiments. This method is suitable for use in factory laboratories. The accuracy was about 1% with small thermos

bottles, but can be increased by using large size bottles. It was found that nearly the same amount of fat crystallized in cream and separated butterfat with winter cream at 18 to 25° C. and with summer cream at 26 to 30° C. both during a day.

The authors arrive at the following conclusions: The dilatometric method is excellent in examining butterfat, but should be wholly abandoned for cream as giving erroneous results. To ascertain the quantity of crystallized fat in cream the new method should be used, which opens a wide perspective, because now for the first time it is possible to control the manufacturing process.

A. F. Tamsma

447. Milk treatment with oxidation-inhibiting gases. M. E. DUNKLEY. U. S. Patent 2,463,363. 2 claims. Mar. 1, 1949. Official Gaz. U. S. Pat. Office, 620, 1: 285. 1949.

Oxidation of such dairy products as butter, evaporated milk, whole milk powder, etc., is prevented by a system of handling all processing of the milk from the milking machine under an atmosphere containing 8.2% carbon dioxide, 0.1 to 0.2% acetylene, 0.1 to 0.2% oxygen, 3.8% carbon monoxide, 2.2% methane and 85.5% nitrogen. This gas is produced by burning fuel gas.

R. Whitaker

448. Fosfataseenzymets Varmedestruktion. (Destruction of the milk phosphatase by heating.) R. HANSEN. Nord. Mejeri Tid., 14, 8: 3-10. 1948.

A new technic in establishing curves of destruction of the milk phosphatase at different temperatures was developed by the State Experiment Station, Denmark. Glass ampules, 100 mm. high, 19-20 mm. in diameter and 0.5 mm. thick were utilized. An injection syringe was used to fill the ampules with enzyme concentrate. Three tables and two graphs are given with temperatures and the number of seconds necessary for the destruction of milk phosphatase.

The following is a summarization of the data:

No. of sec. to destroy phosphatase

Pasteurization temp.	% destruction of enzyme		
	99.6	96.0	90.0
(°C.)	(sec.)	(sec.)	(sec.)
60	3,540	1,740	1,086
63	810	400	256
66	199	100	64.5
68	75.0	38.4	24.6
71	20.5	10.3	6.6
74	5.9	2.95	1.90
77	1.65	0.85	0.55
80	0.44	0.23	0.15

G. H. Wilster

449. Visual observation and silhouette projection apparatus. G. D. AMERDING. (Assigned to Mojonnier Bros. Co.) U. S. Patent 2,461,623. 7 claims. Feb. 15, 1949. Official Gaz. U. S. Pat. Office, 619, 3: 721. 1949.

The image of a Babcock fat test bottle is projected on a diaphragm by means of a beam of parallel light rays in such a manner that the reading of the fat column is facilitated. A heater is provided for maintaining the device at an elevated temperature. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

450. Interessant planl sning av kombinert meieri. (Interesting plan for a multiple-product dairy plant.) ANONYMOUS. Meieriposten, 37, 14: 245-246. Apr., 1948.

To arrange a creamery so it has a practical layout for carrying out all of its processes for all products constitutes a problem which this article attempts to solve. It suggests a star-shaped floor plan. The center of the star plan would contain the platform and separator room. An illustration shows the various star wings housing the equipment for buttermaking, cheesemaking, market milk and one star wing left vacant for future development. Two large storage rooms could be built across the end of one star wing where cheese could be cured. Room is allowed for the boiler in this creamery plan which lends itself to an expanding plant. The star plan allows for plenty of light and ventilation. It has the advantage of having all rooms join the center room which houses the separator and milk handling equipment. Expansion can be accomplished by adding on to the star wings without disrupting the general plant activities. It is as economical to build the star plant building as to build a single building of more than one story, containing the necessary halls and stairways. The plan was developed by A. P. Andersen and I. Nielsen. G. H. Wilster

451. Do's and don'ts on cold-room insulation. ANONYMOUS. Operating Engineer, 2, 1: 36-37. Jan., 1949.

Insulation is used to reduce the heat flow from the warmer to the cooler area to a reasonable amount. After thickness of insulation for a given heat flow is decided upon, building design should be studied to ascertain that the full thickness can be applied everywhere.

Foundation concrete should be poured short to allow sub-floor to overlap wall to make room for full thickness of insulation at sub-floor and

building wall joint. Door sills must be made properly or door may become cause of greatest refrigeration loss. Roof joists placed too closely to the building walls may prevent proper thickness of insulation. Roof flashing also is very important here. Cover building walls with asphalt paint or paper to prevent leaks of moisture-laden air into insulation.

Steps in repairing damaged corkboard are: (a) On masonry walls, smooth surface with cement plaster. (b) When dry, cover with asphalt primer paint. (c) Apply first layer of corkboard with hot asphalt. (d) With additional layers, fasten each board to first layer with hardwood pulp.

Protect insulation with an airproof, moisture-proof and odorless cover. Portland-cement plaster and asphalt plastic may be used. Illustrations present correct design for critical points in cold-room construction. H. L. Mitten, Jr.

452. How to choose economical type of roof construction. H. J. SCHARRES, Graham, Anderson, Probst and White, Architects, Chicago. Heating, Piping Air Conditioning, 21, 3: 93-95. Mar., 1949.

Because each project presents different problems, rules for achieving economy cannot be set to apply to all types of roof construction. Heating and air conditioning designs must be correlated with the rest of the structure. Usually this takes much calculation to produce results which are understood readily by the architect and the client. To make the problem easier tables were prepared for eight of the most-used types of roof decks with seven kinds of insulation. The tables show the construction, heat transfer coefficient for the kind of insulation and thickness selected, thermal conductivity and thermal conductance. H. L. Mitten, Jr.

453. Temperature control system for pasteurizers. J. I. HALL. (Assigned to Ex-Cell-O Corp.) U. S. Patent 2,465,532. 1 claim. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1429. 1949.

This device correctively controls the magnitude of the current applied to milk when it is pasteurized by resistance heating, in spite of variations in flow, composition and initial temperature of the milk. The direct current corresponding to the temperature of the milk downstream from the electrodes is amplified and employed to vary the current between the electrodes.

R. Whitaker

454. Safe application and operation of ammonia equipment in ice cream plants. V. C. PATTERSON, V. C. Patterson & Associates, York, Pa. Rept.

Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 74-78. Oct., 1948; and Ice Cream Trade J., 45, 3: 50, 52, 96, 97. Mar., 1949.

Safety in operation of direct expansion refrigeration requires a knowledge of the properties of ammonia. Ammonia in amounts of 13.1 to 26.8 by volume in air becomes explosive and gas masks are not effective when the concentration reaches 3 to 4%. In the application of safety first practices in ammonia plant designs an operator's attention should be given to the following: (a) Locate condenser, high pressure receiver and charging connections outside building or on roof. (b) Locate compressor room on ground floor, separate from boiler room, with ample exits and good ventilation. (c) Design so all ammonia charge can be transferred to low pressure side while plant is under full operation without liquid slopping over into any compressor. (d) Equip all evaporators with relief valves which open to atmosphere, not into the suction line. (e) A fireman's emergency station located outside the building, equipped with a stop switch for all machinery in compressor room, a transfer valve to dump high pressure ammonia into a low pressure surge drum to relieve all high pressure lines and a hand-operated valve to relieve ammonia pressure into a low temperature surge drum over into a water mixing tank. Definite instructions should be not to dump ammonia unless pressure is either 100 lb. or over, or 200 lb. or over. Other precautions include an emergency fire hose, protected ammonia lines, storage of minimum amounts of ammonia on high side, high pressure cut-outs on all high stage compressors and all lines properly hung, braced and protected against physical damage. A colored floor diagram of the cycle should be framed, covered with glass and hung in compressor room. Ammonia masks and CO₂ fire extinguishers should be located at convenient places and operators trained to handle an emergency. When automatic operation is used, the best plan is to have two small compressors with one operating all the time and one thermostatically controlled coming on and off as required.

W. H. Martin

455. Basic principles of piping (a review of fundamentals). H. VETTER, Consulting Engineer, Los Angeles. Heating, Piping Air Conditioning, 21, 3: 79-82. Mar., 1949.

The difference between steam and refrigeration plants is in the boiling temperatures and application. In refrigeration plants the evaporator coil corresponds to the boiler and the compressor is in the same position as the engine. The piping between an evaporator and engine should be

pitched toward the evaporator so that condensate formed in the line will drain back to the evaporator. When globe valves are placed in horizontal lines (valve stem vertical) the engine side of the line should be tapped and connected to a drip trap to prevent the collection of condensate in the line.

Pipe line size should be determined by desired pressure drop and not by velocity; however, high velocities should be avoided. Equations, graphs and tables are presented which give pressure drops and velocities for steam pipes. An ideal leader arrangement is one in which the pressure drop to each engine (or compressor) is the same. Difference in pressure drop is more important in refrigeration compressors than in steam engines, for compressors do not have a governor to compensate for the difference. A bottleneck in the piping will reduce the capacity of the system. Locate stop valves in the outlets of all pressure vessels so that, in the event of leaks, the pressure can be shut off to allow repairs without shutting down the evaporator.

H. L. Mitten, Jr.

456. Flexible couplings. J. J. O'CONNOR, Power, McGraw-Hill Publishing Co., New York, N. Y. Power, 93, 4: 87-102. Apr., 1949.

The coupling transmits all power between driving and driven units. Couplings should be used to protect against misalignment which occurs under operating conditions. They should not be used to connect two shafts originally out of line.

There are many types of couplings and their applications overlap. This article attempts to explain couplings so that the plant engineer may select, install and operate flexible couplings successfully.

If a machine and its driving unit are in line they usually are connected by a coupling. Since perfect alignment is practically impossible, flexible couplings are necessary. Misalignment of machines connected by rigid couplings causes excessive bearing wear and power consumption.

Use of yielding material or a mechanical design which allows movement between rigid elements are the two general means of obtaining flexibility in couplings. Some couplings provide flexibility in four directions, tangential, angular, radial and axial. Others are limited to two or three directions. The number of directions of flexibility is dependent upon the application.

Proper selection of a coupling requires it to be large enough to fit the shafts, capable of carrying the load and capable of operating under the prevailing plant conditions. Couplings usually are rated in horsepower/100 r.p.m.

One of the most common causes for trouble is the selection of couplings which are too small for the application. Centrifugal pumps and fans cause little wear. A plunger pump subjects the coupling to considerable shock.

H. L. Mitten, Jr.

457. Fuels and firing. Part I. P. SWAIN, J. McCABE, AND B. SKROTZKI, McGraw-Hill Pub. Co., New York, N. Y. *Operating Engineer*, 2, 2: 19-34. Feb., 1949.

Combustion is high-speed oxidation in which the gas generated from the fuels burns, rather than the solid or liquid fuel itself. Yellow flames are caused by the glow of a concentration of individual carbon molecules just before they are burned. If the gaseous mixture containing hydrogen and carbon is cooled, the hydrogen may burn and the carbon may deposit out as soot. When a yellow flame in a boiler is cooled by such things as boiler tubes, unburned carbon is deposited or swept up the stack as smoke. Soot and smoke can be prevented by allowing complete combustion before the flame reaches the tubes. When combustion is complete the hydrogen always ends up as H_2O and the carbon as CO_2 . When the carbon is not burned entirely it may appear in CO , which is capable of further burning.

Coke and charcoal are mostly solid carbon. Bituminous coal contains, in addition, distillate hydrocarbon. Natural gas is almost all gaseous hydrocarbon. Carbureted water gas is largely CO and hydrogen. Fuel oil vaporizes into gaseous hydrocarbon before burning. If cracked, it may produce some solid carbon and hydrogen. Coal decomposes into gaseous hydrocarbon, CO and hydrogen before burning.

Suspension firing of oil and coal requires that each be broken into as many particles as possible. To prevent smoke, combustion steps must be complete during the travel of the fuel from the burner to the furnace outlet. Good combustion depends upon temperature, time and turbulence. In fuel bed firing, volatile matter distills off and coke left in the grate burns to mixture of CO_2 and CO . The gases from the fuel bed are mixed with the air which flows through the grate. Secondary air may be admitted above the grate to furnish oxygen for complete combustion. From 40 to 60% of coal's heat is liberated above the fuel bed. In furnaces, the less excess air used, the larger must be the combustion space to insure complete combustion. Modern furnaces designed to keep size and investment low and efficiency high tend to use high speed air jets for more turbulence.

Natural gas, manufactured gas, blast-furnace

gas, commercial fuel oils and coal are discussed. In designing a new plant a fuel survey should be made to avoid a plant which will prevent a free choice of fuel. Facts needed are (a) fuels available, (b) how available fuels compare now, (c) how they will compare in the future. Don't skimp on equipment. Plan for flexibility in fuel selection.

Combustion chemistry is discussed and a short-cut for combustion calculation is presented.

H. L. Mitten, Jr.

458. How to size your blowoff tanks H. B. NICKELSPORN, Radiant Engineering Co. *Operating Engineer*, 2, 1: 24-25. Jan., 1949.

Blowoff tanks prevent direct discharge of steam-water blow-down mixture into sewer lines which are not built to take blow-down temperatures. Direct discharge into sewers also might cause steam to back up into other equipment which empties into the sewer line. Blowoff tank provides space for blow-down to cool and lose pressure. Flash steam is vented to the atmosphere.

American Society of Mechanical Engineers Code requires blowoff valves to be no larger than 2.5 in., no smaller than 1 in. Valves such as globe valves having pockets in which sediment might collect cannot be used. All valves and fittings must have American Standards Association rating 25% higher than boiler safety-valve setting. When pressure exceeds 100 psi. each blow-down must have two valves in series. One table shows per cent of flash steam in blow-down at various temperatures and another gives vent and discharge sizes.

Blowoff tank size is dependent upon the weight and volume of blow-down mixture at a single period. A tank volume equal to $1/5$ the water-holding capacity of the boiler or water volume of one gauge on water column may be used. Boiler pressure does not affect tank size. Allow for a cold water seal to remain in the tank between blow-downs.

Tank design must be approved by local authorities. As a guide use A.S.M.E. Code for unfired vessels. Tank should be designed to withstand half the boiler pressure. Tanks may be vertical or horizontal. Vent line must be twice inlet size. Making vent larger than necessary causes less water to be carried out by flash steam. No valves or other restrictions should be in the vent line. Line must rise 7 ft. above places where people may walk. Discharge lines must be twice the area of the blowoff pipe and must have an elbow inside the tank at least 6 in. from bottom. The discharge line should have a siphon breaker at the highest point.

H. L. Mitten, Jr.

459. Refrigerating apparatus including hydraulic lift. S. G. PRICE. U. S. Patent 2,463,307. 4 claims. Mar. 1, 1949. Official Gaz. U. S. Pat. Office, 620, 1: 270. 1949.

To facilitate insertion and removal of cans of milk or other perishable food products in a tank of cooling liquid, a platform is raised from or lowered to the floor of the tank by hydraulic means. R. Whitaker

Also see abs. no. 418, 419, 420, 427, 466, 467.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

460. Controlling fat losses. L. C. THOMSEN, Univ. of Wisconsin, Madison. Milk Dealer, 38, 6: 52, 126-134. Mar., 1949.

Data are presented showing the causes of fat losses in dairy plants. Hidden losses result from the use of wrong conversion factors in converting from volume to weight or vice versa, from incorrect test samples, incorrect testing procedures, or when one testing procedure is to be reconciled with another. Apparent losses include spillage, spoilage, incorrect standardization for composition, and larceny. In the control of losses it is suggested that all employees should be made waste and loss conscious by showing the cash value of losses and then offering bonuses for keeping them within pre-established limits. C. J. Babcock

Also see abs. no. 475.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

461. Measurement of sperm activity before artificial insemination. LORD ROTHSCHILD. Nature, 163, 4140: 358. 1949.

This method is based on the observation that semen, when active, exhibits well-defined changes in electrical impedance. A conventional alternating current bridge, energized by a 5 kc. oscillator, is used for measuring the electrical changes. The detector is an oscilloscope with an amplifier. The unknown arm of the bridge consists of a pair of platinized platinum electrodes which dip into the semen at 37° C. The voltage across it is about 50 m.v. The frequency is greatest when first collected, decreasing with temperature and reading zero when the sperm are dead or feebly motile. Phosphate buffer added to the semen maintains the initial frequency. The impedance is increased greatly by concentration of the semen, i.e., centrifugation. R. Whitaker

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

462. Cutting milk production costs with pen barns. CLARON BURNETT, Assoc. Ed. Milk Dealer, 38, 6: 42-43, 112-116. Mar., 1949.

Comparisons of pen type stabling of cows with the conventional stanchion barn at the University of Wisconsin experimental farm indicate more milk can be produced with less labor and lower feed costs when the newer type barns and milking parlors are used. Studies at the Huntley, Montana, experiment station show cows produce as much as 19% more milk and 18% more butterfat when housed in pen barns than when kept in a stanchion barn. Studies of the two types of barns at Wisconsin have shown: (a) Over-all labor requirements have run about 10% less for pen barn operations than for the stanchion barn. (b) Cows housed in pen barns have better appetites and consume more roughage with the result that cost/lb. of 4% fat-corrected milk was only 96.11% of that produced by cows in the stanchion barn. (c) There is little difference in the quality of the milk as shown by bacterial counts between the individual barns. (d) Cows have remained healthier and there has been less trouble with mastitis in pen barns. Other advantages of pen barns include economy of construction, ample fresh air and sunshine for cows and better-preserved manure for application to the soil. Disadvantages include larger bedding requirements and the necessity of dehorning the cows. C. J. Babcock

463. Insecticide studies with dairy cattle. L. A. MOORE, R. H. CARTER AND F. W. POOS, U. S. Dept. of Agr., Washington. J. Milk and Food Technol., 12, 2: 103-104. Mar.-Apr., 1949.

The authors indicate the danger of using DDT to control insects on crops fed to cows and the milk containing appreciable quantities of the insecticide. However, if the amounts of DDT are kept to a minimum for the control of the insects, no large amount of this compound will appear in the milk.

No doubt, other insecticides will be developed that will be just as effective as DDT in controlling insects and will not be absorbed and secreted in the milk as readily. H. H. Weiser

464. Teat cup. A. C. WEIBY. (Assigned to Solar Corp.) U. S. Patent 2,462,583. 3 claims. Feb. 22, 1949. Official Gaz. U. S. Pat. Office, 619, 4: 1096. 1949.

This milking machine teat cup consists of a rigid metal outer shell, within which and re-

leasably secured to it is a flexible tube molded to receive the teat. Intermittent suction contracts the tube, withdraws the milk and discharges it to the milker through a tube attached to the lower end of the outer shell. R. Whitaker

465. Alarm device for milking machines. A. G. PERKINS. U. S. Patent 2,461,439. 8 claims. Feb. 8, 1949. Official Gaz. U. S. Pat. Office, 619, 2: 567. 1949.

A receptacle is mounted inside the milking machine pail directly under the ports which deliver the milk from the cow. This receptacle is lowered as it fills with milk and stays down as long as there is a full flow of milk. When the flow decreases at the completion of the milking the receptacle rises, thereby sounding an alarm and finally breaking the vacuum if the attendant does not remove the milker from the cow when the alarm is sounding. The rise and fall of the receptacle is governed by a drain which discharges the milk from the receptacle at a slower rate than the normal milk flow but faster than the reduced flow toward the end of the milking.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

466. Short time high temperature pasteurization of ice cream mixes. C. M. MINTHORN, Chester Dairy Supply Co., Chester, Pa. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 47-50. Oct., 1948.

Mix prepared from concentrated products must be preheated to 125° F. before it enters the first section of the Ste-Vac heater. Condensed mix can be pumped directly from the vacuum pan through this heater where the temperature is raised to 157° F. At this point, the mix is homogenized. Friction raises the temperature to 162° F. and the mix is pumped through the second section of the heater. It enters the holding tube at 175° F and is held 23 sec. before it is cooled. Bacterial counts in mix pasteurized by this method compare favorably with counts in mix pasteurized by the batch method.

H. B. Naylor

467. The use of the vacreator for high temperature pasteurization of ice cream mixes. G. H. WILSTER, Oregon State College, Corvallis. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 24-40. Oct., 1948.

The use of the vacreator for pasteurizing cream and ice cream mix and the application of the vacreator for condensing milk and ice cream mix

was discussed. Mixes pasteurized by the vacreator process had lower bacterial counts, lower viscosities, better flavor and better whipping properties than control mixes pasteurized by the vat method. The finished ice cream had a better body and fresher flavor than ice cream from the vat-pasteurized mixes.

H. B. Naylor

468. Some factors influencing shrinkage in ice cream. J. J. SHEURING, Univ. of Georgia, Athens. Ice Cream Rev., 32, 8: 44, 129-133. Mar. 1949.

Factors studied which were found to increase the amount of vacuum-induced shrinkage of batch-frozen ice cream were: (a) Increasing the fat content of the mix, particularly within the range of 12 to 15% fat. (b) Use of sweet cream as compared with similar mixes prepared with butter or frozen cream. (c) Neutralization of high acid mixes with either sodium bicarbonate or magnesium carbonate increased the amount of shrinkage over that observed with low acid mixes to which no neutralizing agent had been added. (d) Reconstituting a dehydrated mix and freezing immediately resulted in more shrinkage than when reconstituted mixes were aged overnight prior to freezing. Storage of dehydrated mix for a period of 40 d. had no significant effect on shrinkage when reconstituted, provided the reconstituted mix was aged overnight before freezing. (e) Freezing of unaged mixes was found to increase the amount of shrinkage; however, no advantage was observed in aging mixes longer than 48 hr.

Any factor which will tend to produce large air cells and large ice crystals will reduce shrinkage, whereas small air cells and small ice crystals will increase shrinkage in ice cream.

W. J. Caulfield

469. Forsøg med Emulgatoren "Creminovo" i Flødeis. (Experiments on the emulsifying agent "Creminovo" for ice cream purposes.) English summary. O. S. HANSEN, Ladelund. Nord. Mejeri Tid., 14, 4: 8-10. 1948.

Experiments in ice cream making have been undertaken with a new synthetic emulsifying agent "Creminovo" manufactured by Emulsion, Ltd., Juelsminde. Creminovo is a fine, very light, yellowish powder resembling egg yolk and, like the latter, containing about 55% fat. It is easily soluble in water at 60-70° C. but possible clots cannot be stirred easily into the ice cream compound any more than clots of milk powder and egg yolk, for which reason Creminovo preferably should be mixed with milk powder, sugar and gelatin before being stirred into the ice cream compound. By such procedure it is dissolved easily at a temperature of 65-70° C. within 10

to 20 min. Creminovo increased the viscosity and whipping ability of the compound and produced an ice cream that was less watery and "cold" and had a more velvety and fat consistency. The effect was in all respects equal to that of the same quantities of egg yolk and an emulsifying agent.
G. H. Wilster

470. The use of emulsifiers in ice cream P. H. TRACY, Univ. of Illinois, Urbana. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 53-67. Oct., 1948.

See abs. no. 376, p. A78.

471. Controlling viscosity in chocolate ice cream mixes. C. D. DAHLE, W. R. DAVEY AND W. D. SWOPE, Pennsylvania State College, State College. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 5-14. Oct., 1948.

The results of studies dealing with the effect of processing methods, type of stabilizer and type of cocoa on the viscosity of chocolate mixes were presented.

When gelatin was used as the stabilizer, mixes flavored with domestic cocoa had much higher viscosities than mixes flavored with Dutch cocoa. The viscosity of mixes containing domestic cocoa could be controlled satisfactorily by reducing the homogenizing pressure or by standardizing the acidity with sodium bicarbonate. Raising the homogenizing temperature increased the viscosity somewhat with both types of cocoa.

Dariloid as the stabilizer usually caused excessive viscosity when the mixes contained Dutch process cocoa. This condition was not corrected by standardization of mix acidity with sodium bicarbonate, or by the addition of stabilizing salts. The viscosity of mixes containing domestic cocoa was not excessive, but increased when soda or a stabilizing salt was added.

Cellulose gum gave results similar to those obtained with Dariloid. Mixes containing Dutch process cocoa had extremely high viscosities when this stabilizer was used. A satisfactory mix could be prepared using domestic cocoa. Chocolate mixes require less stabilizer than plain mixes, due to higher total solids content.
H. B. Naylor

472. Galliker packs pints in bowls in a premium promotion. ANONYMOUS. Ice Cream Trade J., 45, 3: 42, 85, 86. Mar., 1949.

Premium promotion as a means of selling ice cream has been used successfully by the Galliker Dairy Co., Johnstown, Pa. The premium consists of a kitchen bowl. The color of the bowl and flavor of ice cream are changed monthly. The wholesale price to dealers for this item is

\$1.76 per gallon, the retail price to consumers is 37 cents per pint. The dairy pays 6 cents for the bowl and 22 cents for the ice cream less carton cost. The bowls are packed in a 5.5 x 5.5 x 2.625 in. knock-down style carton, which eliminates wrapping at the store.
W. H. Martin

473. Current sales trends. ANONYMOUS. Ice Cream Trade J., 45, 3: 46, 88. Mar., 1949.

Jan., 1949, ice cream sales were about 4% below Jan., 1948, for the country as a whole. Areas hit by heavy snow showed a loss of about 14% and the west coast was down 33%. Some areas in the east had slight losses and some areas showed a gain in Jan. sales.
W. H. Martin

474. Supermarkets. M. M. ZIMMERMAN, Editor and Publisher, Super Market Merchandising. Ice Cream Trade J., 45, 3: 74, 104-106. Mar., 1949.

Supermarkets have increased from 1,200 in 1936 to 12,000 in 1948, doing nearly 30% of the grocery store volume, with an average store volume of \$643,000 per yr. Stores attract from 25,000 to 50,000 customers per wk. The average gross mark-up is 17 cents on the dollar and the average net, 1 to 2 cents on the dollar. Eighteen hundred to 3,000 items, each in its own package, are handled. Markets with two to four frozen food cases for dairy foods are common. Under such conditions the style, design and type of package is of great importance in merchandising ice cream.
W. H. Martin

Also see abs no. 441, 454.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

475. Three day delivery. ANONYMOUS. Milk Dealer, 38, 6: 76-78. Mar., 1949.

Indianapolis dealers operating under the 3-day delivery plan report as follows: On wholesale sales, three had an increase up to as high as 9.9%, one had a decrease and one made no comment. On retail sales, five indicated increases up to as high as 7.2%, three reported no change and two had decreases. On gasoline the average was a saving of 6.1%, with a low of 2 and a high of 12.6%. On manpower, seven saved no men and three did save men. Average increase in bottles in service under the 3-day system (nine dealers) was 41%, and average increase in bottle cases in service was 36%. Nine dealers advised no hardship was inflicted in the plant under the new system and eight reported it caused no overtime. Six said it required no additional cooler space while two said it did.
C. J. Babcock.

476. Milk strainer. E. ZIKA. U. S. Patent 2,465,623. 1 claim. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1453. 1949.

This strainer, designed for placement on a milk can, has a throat which has a flat perforated plate for supporting the filter medium. The outside of the throat is tapered and fits snugly into the neck of the can. Vacuum is applied to the can through a tube built in the strainer which hastens the rate of straining. R. Whitaker

477. Tote box. C. W. PRAEGER, H. BLUM AND H. JOCHIMSEN. (Assigned to Sturdibilt Milk Box Corp.) U. S. Patent 2,464,343. Mar. 15, 1949. Official Gaz. U. S. Pat. Office, 620, 3: 840. 1949.

A box for holding milk bottles and paper cartons of milk for convenient handling in milk plants and on delivery vehicles is described. The upper edges of the ends of the box are indented and bent outward to facilitate handling. R. Whitaker

478. Apparatus for collecting liquid sediments. N. FERRAEZ. U. S. Patent 2,463,481. 4 claims. Mar. 1, 1949. Official Gaz. U. S. Pat. Office, 620, 1: 313. 1949.

This device is designed to draw quickly and easily a sample of milk from the bottom of a can and pass it through a sediment pad. The apparatus is operated by vacuum which is applied by actuating a foot-operated valve. R. Whitaker

479. Tillsats av bikarbonat till homogeniserad grädde. (The addition of sodium bicarbonate to homogenized cream.) ANONYMOUS. Mejeritekniska Medd., No. 5-6: 83. Dec., 1948.

Homogenization stabilizes the dispersion properties of fat so that the formation of a cream plug is hindered. At the same time the stability of the protein is reduced and coagulation is favored. As coffee contains a substance that favors coagulation, the homogenized cream sometimes is curdled when added to coffee. To prevent this, about 30 g. NaHCO_3 can be added per 100 l. cream. Citrate or Na_2HPO_4 also can be used for this purpose. G. H. Wilster

Also see abs. no. 432, 435, 436, 437, 438, 482.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

480. Forsatte undersøgelser over innholdet av A-vitamin og karotin i norsk smør og mjølk. (Further investigations on the content of vitamin A and carotene in Norwegian milk and butter.)

English summary. HARALD HVIDSTEN, LIS GILBE HANSTEEN AND GERD BROCH. Meieriposten, 37, 11: 186-192. Mar., 1948.

As a supplement to earlier investigations, some determinations of carotene and vitamin A in 33 samples of butter from cow's milk, five from goat's milk and two from sheep's milk have been carried out. The carotene content of some of the feeding stuffs used for the production of the butter samples also has been determined. The determinations of carotene and vitamin A have been effected by photometric methods. The samples of butter from cow's milk stem partly from pasture (cultivated pasture and highland pasture) and partly from barn feeding, in which the carotene content of the daily ration ranges from 33 to 1,360 mg.

The carotene content of butter samples varied from 1.2 to 7.6 γ/g . of butterfat (2-12.7 I.U. vitamin A), the vitamin A content from 14 to 32 I.U. and total computed content of vitamin A (vitamin A effect) from 16 to 42 I.U./g. of butterfat. The following correlation was found between mg. of carotene in the daily ration (x) and: (a) total I.U. of vitamin A effect per g. of butterfat (Y): $Y = 20.7 + 0.0129 x$; $r = 0.69$; (b) total I.U. of vitamin A effect in milk per day (Y): $Y = 10200 + 8.6 x$; $r = 0.78$; (c) the vitamin A effect in daily milk yield in % of the daily carotene content in feed (Y): $\log Y = 2.2074 + 0.7204 \log x$.

In butter from goat's milk, 40 to 66 I.U. of vitamin A were found per g. of butterfat. The carotene content was small. In butter from milk of sheep, 30 I.U. of vitamin A were found per g. of butterfat.

Determinations of carotene and vitamin A directly in milk have given results which were in good agreement with the determinations in butter. G. H. Wilster

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

481. The sterilization of milk bottles at farms and dairies. S. B. THOMAS, Natl. Agr. Advisory Serv., Crosswood, D. GRIFFITHS, T. LEWIS, K. J. MORGAN, AND E. P. DAVIES, Natl. Milk Testing Serv., Brynawel, Aberystwyth. Proc. Soc. Applied Bact., 1947, 1: 6-12. 1947.

A large number of bottles examined after hand washing, washing by rotary spray machines or straight-through machines revealed that a large number contained over 600 bacteria per bottle, a number which was "unsatisfactory" according to prescribed standards. Causes for the high counts usually were the result of using incorrect

alkalinity of insufficient time in and temperature of the detergent. Dairies using steam sterilization showed lower counts on bottles than those using chlorine.

M. L. Speck

482. Seasonal variation in the sterility of washed milk churns. G. ELLIS JONES, Natl. Agr. Advisory Serv., Crosswood, Aberystwyth. Proc. Soc. Applied Bact., 1947, 1: 13-14. 1947.

During a 4-year study, a peak in the number of bacteriologically unsatisfactory milk cans (rinse count > 250,00 when dry or > 50,000 when wet) occurred during June-Sept. A minor peak also was observed in Feb., this peak becoming less marked with improved sanitation. The following were considered causes for the summer peak: (a) higher bacterial content of the milk residues left in the cans during the warmer weather; (b) less careful washing during the period of maximum milk intake at the dairies; (c) shortage of milk cans during the rush season which led to the use of old, dented and rusty cans; (d) the use in some instances of old and inefficient can washers which are brought to use in the rush season. The winter peak was thought to be caused by the higher thermoduric bacterial content of milk as reported by some to be present in winter.

M. L. Speck

483. We have made progress. RAY TARDIFF, Breyer Ice Cream Co., Philadelphia, Pa. Ice Cream Trade J., 45, 3: 89-92. Mar., 1949.

The dairy industry committee has made much progress in plans to standardize all equipment used for the production, transportation and processing of milk so that it is easy to clean, operate and maintain. Standards for equipment are developed and submitted to the Dairy Industry Committee for approval and then to the Committee on Sanitary Procedures of the International Association of Milk and Food Sanitarians and to the Milk and Food Unit of the U. S. Publ. Health Service. Then a joint meeting of these three groups is held to arrive at standards.

Standards have been developed for centrifugal and positive rotary pumps, sanitary fittings, storage tanks, weigh cans and receiving tanks for raw milk. Tentative standards are being considered for milk transportation tanks, can washers, homogenizers and high pressure pumps, gauges for milk storage tanks, dairy ware, milk pails, strainers, milking machines, electric motors, plant heat exchangers, surface type heat exchangers and

tubular type heat exchangers. Work also has been started on cabinets and soda fountains.

W. H. Martin

484. Studies of quaternaries as bactericides. G. A. WEBER, U. S. Pub. Health Service, Cincinnati, O. J. Milk and Food Technol., 12, 2: 107. Mar.-Apr., 1949.

More information on the quaternary compounds as bactericides is urged by the author. Since these compounds are surface-active, they are inactivated readily by anionic agents such as soaps and synthetic detergents. The amphoteric nature of protein foods enhances their absorption by forming a film over the surfaces, thus protecting the bacteria beneath the film. Anionic agents can break up this film readily.

In order to evaluate the germicidal efficiency of quaternary compounds, more satisfactory activators are needed as compared to those used on chlorine. The author suggests additional studies, such as the efficiency of quaternaries against pathogenic bacteria and viruses, a chemical measure for effective quaternary residual, more information on interfering substances in natural waters, the longevity of the germicides in the rinse vat and suitable concentration levels for adequate disinfection.

H. H. Weiser

485. Studies on high temperature dishwashing. W. L. MALLMAN AND D. KAHLER, Dept. of Bact. and Pub. Health, Michigan State College, East Lansing. J. Milk and Food Technol., 12, 2: 105. Mar.-Apr., 1949.

The destruction of *Micrococcus caseolyticus* used as the test organism was made on four leading makes of single-tank conveyor curtain rinse dishwashing machines. The authors suggest that anything less than 99.5% kill of the organism studied failed to meet this requirement. A minimum standard of performance is (a) temperature, 170° F., (b) exposure, 10 sec., (c) 1.5 gal. of water per 20 in. tray or 9 gal. per min.

Single-tank machines should maintain a wash temperature of 160° F. and rinse at 170° F. for maximum efficiency. In other types of machines equipped with rinses, the temperature should be set at 170° F. for 10 sec. or held at a range between 140 and 160° F.

H. H. Weiser

486. Insect and rodent control. E. M. SEARLS, Sealtest, Inc., New York, N. Y. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., 2: 87-94. Oct., 1948.

See abs. no. 414, p. A85.

Also see abs. no. 435, 437, 478.

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEWS

487. **Farm work simplification.** LAWRENCE M. VAUGHAN and LOWELL S. HARDIN. John Wiley and Sons, Inc., New York, N. Y. 145 pp. \$2.80. 1949.

This book deals with the simplification of practically all phases of farm work. It is broad in its scope and sets forth the principles of effective work from the standpoint of labor, machines, organization and the human factors involved in the simplification of the usual farm tasks. Several pages are devoted exclusively to time- and labor-saving on dairy chores and specific examples with reference to milking operations, barn arrangement, feeding practices, etc., that will reduce labor are set forth.

The book is divided into two parts. Part I discusses the place of work simplification in farming with respect to savings that can be made, the principles of effective work and how the results may be applied. Part II is devoted to methods and plans that may be used in making an analysis of any operation from the standpoint of developing time- and labor-saving methods. Such items as product analysis, man analysis, man and machine analysis and body motions are stressed. Suggestions for teaching and training individuals in work simplification are stressed with emphasis on the fact that research and education go together.

Throughout the book are found examples of labor savings for specific jobs that well may be adapted to most farms seeking to improve their efficiency. Since dairy farming involves not only feed and care of cattle but crop production, hay harvesting, transportation, etc., this book is well worthwhile for the practical operator, the plant fieldman, extension workers, research economists and the teacher of agriculture.

H. A. Herman

488. **Animal breeding.** LAURENCE M. WINTERS. 4th ed. John Wiley and Sons, Inc., New York, N. Y.; Chapman and Hall, Ltd., London. 404 pp. \$4.50. 1948.

Keeping pace with the progress in animal breeding through the years since the first edition of this book in 1925, the present fourth edition again has been expanded and changed. Numerous additions have been made to include many recent developments and more detail in this well-known book. The opening chapter has been expanded and now contains an introduction to the problem of animal improvement by breeding. Among the several reorganizational changes has been that of making gametogenesis serve as the connecting link between the chapter on "The Reproductive Organs" and the chapter on "The Physical Basis of Heredity". The order of presentation of much of the applied material has been changed to follow the more fundamental sections of the book. New chapters entitled "The Effectiveness of Selection" and "Building Superior Germ Plasma" have been added. The author cites many examples from his own extensive experience in breeding research, as well as from the research of others, to illustrate the application of the fundamental principles of breeding to practical animal improvement.

N. L. VanDemark

489. **Milchwirtschaftliche Patentberichte. (Patents concerning the dairy field.)** M. SCHULZ. Volkswirtschaftlicher Verlag Dr. Anton Fehr, Kempten-Allgäu, Germany. 336 pp. 1947.

This book is a compilation of patent reports concerning the whole field of dairy manufacturing processes, both German and foreign, covering the patents for the years 1935 to 1945.

I. Peters

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

490. **Q fever. Experimental Q fever in cattle.** E. J. BELL, R. R. PARKER, and H. G. STOENNER, U. S. P. H., Hamilton, Mont. Am. J. Pub. Health, 39, 4: 478-484. Apr., 1949.

Q fever infection was produced in lactating cows by inoculating the udder with massive doses of a yolk sac culture of *Coxiella burnetii*. The organisms were present in the milk for a long

period of time following injection via the teat canal and in one instance were found for over 200 d. in milk from a quarter inoculated via the glandular tissue. Inoculation of the udder produced an acute mastitis with a marked systemic reaction of brief duration. Recovery from the acute phase was spontaneous with the major symptoms disappearing in about 8 d. Various tissues obtained from these cows sacrificed at intervals up to 63 d. after injection were infectious to guinea pigs. The rickettsiae were recovered from the feces of calves feeding on infected milk. Less success was found in attempting to infect heifers by other routes, such as intranasal, intravenous, by way of the alimentary tract and by way of the vaginal tract. Attempts also were made to transmit Q fever to cattle by infected ticks of the species *Otobius megnini* Duges, but no definite results were reported. D. D. Deane

491. A rapid ring test for brucellosis in fresh milk. G. C. VAN DRIMMELEN, Institute of Onderstepoort, Pretoria, S. Africa. J. S. African Vet. Med. Assoc., 19, 4: 130-134. Dec., 1948.

A colored antigen test for milk samples in the diagnosis of bovine brucellosis is described. The antigen used in the test is stained with haematoxylin. A well-mixed sample of milk is placed in a small test tube and one drop of the antigen is added and mixed well. The sample then is incubated at 37° C. for 50 min. A ring of violet-colored bacilli in the cream layer, with a white milk layer underneath, indicates a positive test. When a white cream layer covers milk which has a violet tinge, the results indicate a negative test. A method for preparing the stain and antigen is outlined. The author reported that the test was in wide use in Denmark and that it could be used to advantage in survey work. K. M. Dunn

492. Pathogenesis of bovine mastitis. I. The relation of age to streptococcal infection. G. R. SPENCER and M. E. KRAFT. Univ. of Wisconsin, Madison. Am. J. Vet. Research, 10, 35: 115-118. Apr., 1949.

The incidence of streptococcal infection is reported according to age for 12 herds in which chemotherapy had not been practiced. Two herds considered to have good sanitary milking management showed a progressive increase from 5.26 to 35% during the first four lactation periods, followed by a decline. Ten herds with poor milking management showed an increase from 60.61 to 72.73% during the first five lactations, followed by a sharp increase. Data from one herd showed that the incidence of infection was also linear during the first lactation. Large herds and poor sanitary practices were associated with high infection incidence. Evidence indicates that

degree of exposure outweighs aging of the udder as a cause of increased infection with successive lactations. E. W. Swanson

Also see abs. no. 532.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

493. Globular and free fat in butter. I. A method for counting and measuring the fat globules in butter and application to the working process. N. KING. Netherlands Milk Dairy J., 1, 1: 19-32. Jan., 1947.

The counting technic developed is as follows: approximately 10 mg. of butter accurately weighed were diluted with 200-500 mg. of butter oil. A very cautious mixing procedure was followed under carefully controlled temperature conditions. A measured quantity of diluted butter was put on a special slide and examined in polarized light by means of a simple mountable polarizer and analyser. The amount of globular fat decreased in the course of working. This decrease was in the number of fat globules rather than a change in the average size. The disappearance of "unbound" moisture was parallel at first with the destruction of fat globules. However, in the last stages of working, the disappearance of the "unbound" moisture was not attended with a destruction of the fat globules. W. M. Roberts

494. Globular and free fat in butter. II. Some applications of the dilution method for determining the globular fat in butter. N. KING. Netherlands Milk Dairy J., 1, 2: 115-117. Apr., 1947.

Application of the dilution method for counting and measuring the fat globules showed that whey butter contained a low percentage of globular fat and that the fat globules were distorted. There was scarcely any difference between the butter from the usual wooden and the metal churn. Also, the fat globules in butter prepared by the Fritz continuous process are deformed appreciably by the strong agitation of the beating wings of this machine. W. M. Roberts

495. Butter workmanship. L. C. THOMSEN. Univ. of Wisconsin, Madison. Natl. Butter Cheese J., 40, 5: 34-35, 58. May, 1949.

The body and texture of butter are influenced greatly by the physical state of the butterfat in the cream and butter. Butterfat exists as free fat—fats with low melting points usually in the liquid state—and as globular fat. The quantity of free fat may be as low as 10 to 15% at the start of the working process and 70 to 75% at the end. A high ratio of free fat to globular fat is respon-

sible for stickiness, leakiness and oiliness in butter. Crumbliness, brittleness and graininess are due to a low free fat-globular fat ratio.

Pasteurization of cream by direct steam injection and churning at high speed mutilate the fat globules, thereby causing an increase in free fat. Low temperature churning and low temperature wash water have an opposite effect. Control of the free fat-globular fat ratio results in control of the body and texture of butter.

H. E. Calbert

496. Experiments on the packing and storage of butter. VI. The effect on keeping quality of butter of exposure to light during manufacture. C. R. BARNICOAT, Dairy Research Inst., (N. Z.), Palmerston North. New Zealand J. Sci. Technol., **29A**, 4: 185-191. Dec., 1947.

Results with 36 well-manufactured butters (of low Cu and Fe content) churned from pasteurized sweet cream and exposed before working to different light intensities for various periods indicated that interior diffuse daylight, not exceeding 50 foot candles and for not over 2-hr. duration, had no deleterious effects on the product.

W. C. Frazier

497. Experiments on the packing and storage of butter. VII. The effect of "free" air in butter. C. R. BARNICOAT, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., **29A**, 4: 193-197. Dec., 1947.

"Free" air content of 16 samples of factory butter varied from 4.9 to 6.7 per cent. Reduction of the air content or working under a high vacuum did not alter the keeping quality but did change appearance and physical characteristics.

W. C. Frazier

498. Experiments on the packing and storage of butter. VIII. The effect of certain added substances on the storage-life of butter. C. R. BARNICOAT, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., **29A**, 4: 199-205. Dec., 1947.

The following substances were added either singly or in combination: (a) dairy salt, (b) borates, (c) citrates, (d) phosphates, (e) oat flour and (f) NaHCO_3 to an alkaline pH. Except with citrates, the results under the conditions employed were discouraging in that the storage life was not lengthened and even seemed to be shortened in some instances.

W. C. Frazier

499. Butter packaging. J. M. NESBITT. Can. Dairy Ice Cream J., **28**, 2: 56-60. Feb., 1949.

A good packaging material for print butter should have the following characteristics: (a) im-

permeability to water vapor, gases, odors and light; (b) close adherence to the surface of the butter to prevent evaporation and loss of weight; (c) grease-proof, odorless and tasteless material; (d) high tensile strength when damp or wet; (e) adequate protection against mold growth on the surface of the butter; (f) easy adaptation to automatic packaging machines and (g) attractive appearance and reasonable cost. The article gives the advantages and disadvantages of aluminum foil, vegetable parchment and fiber boxes.

H. Pynson

500. A cream improvement program. C. H. P. KILLICK. Can. Dairy Ice Cream J., **28**, 2: 40-43, 90. Feb., 1949.

The reasons for the improvement in quality of cream in Manitoba during the past 25 yr. are: (a) the closing of cream-buying stations and (b) the establishment of a compulsory cream grading service with fixed standards and legal minimum price differentials. A price differential between grades of cream is essential as an incentive for improved quality, but if fixed, exerts less effect as the price for butterfat rises. It also was found that the price level for butterfat appears to affect quality. The improvement in quality has reduced off-flavored, old and sour cream to less than 2% and improved butter quality from 70% first grade to 94% first grade.

H. Pynson

501. Four-day grading and its effect on cream quality. H. W. GREGORY, Purdue Univ., Lafayette, Ind. Natl. Butter Cheese J., **40**, 5: 38, 40. May, 1949.

Successful cream grading programs have one or more definite factors required in grading to supplement the senses of taste and smell. Delivery within 4 d. after production serves as a means of insuring a better quality cream. The 4-d. plan of cream grading has brought about an improvement in Indiana butter manufactured from cream purchased under this plan.

H. E. Calbert

Also see abs. no. 519, 533, 534, 535, 568.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

502. Lipolytic bacteria, a cause of rancidity in cheddar cheese. E. G. HOOD, C. A. GIBSON and J. F. BOWEN. Can. Dairy Ice Cream J., **28**, 2: 27-30, 88. Feb., 1949.

An analysis of the results of 16 experimental vats of cheddar cheese made from milk with the addition of varying percentages of lipolytic bacteria gave consistently lower flavor scores and consistently higher acid values than uninoculated vats. Normal uninoculated vats had an average

acid value of 1.0 while inoculated vats had acid values ranging from 1.8 to 16.0 after storage for 10 d. The defects encountered in grading, namely "not clean", "slightly rancid", and "rancid", were the same as those found in commercial grading. The extent to which flavor defects developed and acid degree values increased was related directly to the percentage of lipolytic organisms added to the cheese milk supply. The low flavor scores and the high acid degree values of the experimental cheese were thought to be due to lipolysis or a chemical break-down of the fat as a result of the action of lipolytic bacteria. The presence of lipolytic bacteria suggests that these organisms may be a cause of the sporadic or scattered occurrence of rancid flavor in commercial cheddar cheese.

H. Pyenson

503. Further studies on rheological properties of cheese during manufacture and ripening. Part I. MARGARET BARON. Natl. Inst. for Research in Dairying, Shinfield, Nr. Reading. Dairy Ind., 14, 2: 146-151. Feb., 1949.

Investigations were carried out to determine the relationship between the physical, chemical and bacteriological properties of milk, curd and cheese. The pH of the soft-pitched curd was low in comparison with the rest, with one exception. The streptococci in these curds contained a high proportion of strains capable of producing slight gas in litmus milk. During ripening at 58° F., the cheese firmed up rapidly during the first 14 d., after which they softened somewhat at 28 d. and then gradually increased in firmness at a steadily declining rate until about 130 d. old, after which the body remained constant. The firmness of a cheese throughout its life was related directly to its firmness at pitching.

A highly significant relationship was found between the weight and firmness of green cheese. The heavier the cheese, the softer it was, but this relationship did not exist as the cheese aged.

A detailed study of variation of body throughout a single cheese was carried out on some 28 cheeses. At the top or bottom of the cheese the periphery was slightly firmer than the center.

G. H. Watrous, Jr.

504. Further studies on rheological properties of cheese during manufacture and ripening. Part II. Statistical results. MARGARET BARON. Dairy Ind., 14, 3: 255-263. Mar., 1949.

This report contains a statistical analysis of the work reported in part I of this study. The effects of variations in manufacture on the properties of green cheese were seen most clearly at 14 d. Milk with high fat content gave rise to soft young cheese, and the reverse held true. Milk with high S.N.F. produced a firm, tough cheese when ripe.

High casein content alone did not make cheese firmer than normal.

The firmness at cutting influenced the elasticity of the cheese; curd cut when soft resulted in cheese of high elasticity. The firmness at pitching mainly determined the firmness of the finished cheese; a curd pitched firm was found to cause a firm and dry cheese. The acidity at milling affected the elasticity and possibly the firmness of the cheese; high acidity produced a soft cheese with very little springiness.

G. H. Watrous, Jr.

505. Extraneous matter in cheese. D. C. BECKETT. Can. Dairy Ice Cream J., 28, 3: 96-98. Mar., 1949.

For the third year in succession, extraneous matter tests on cheddar cheese have been conducted in the Dairy School Laboratories at Kemptville during the months of June, July and August. During the 1948 season, a total of 1,407 samples were received for testing. The percentage of acceptable samples was 26.15 in 1943, 65.00 in 1946, 57.72 in 1947 and 86.86 in 1948.

H. Pyenson

506. De stremkrachtbepaling volgens de methode van Van Dam. (The rennet test according to the direct method of Van Dam.) English summary. H. MULDER and L. RADEMA. Netherlands Milk and Dairy J., 1, 2: 128-133. Apr., 1947.

The parallelism found by Van Dam between the peptic and the coagulating activity of rennets does not exist if the rennets contain very different amounts of pepsin. In order to estimate the curdling power of a rennet, it is recommended to estimate first the strength of a chymosin solution according to the method of Van Dam. Then the curdling power of the rennet of unknown strength can be estimated by means of comparative tests. Details of the method are given.

W. M. Roberts

507. Package and packaging material therefor. D. B. ANDREWS. (Assigned to Marathon Corp.) U. S. Patent 2,467,875. 6 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, 621, 3: 889. 1949.

A block of cheese is wrapped tightly in a 2-layer wrapper with a tearing element imbedded in the coating. The wrapper may be removed easily by pulling a tab which causes the material to tear in a predetermined manner.

R. Whitaker

508. Cheese-cutting device. H. A. OLANDER. U. S. Patent 2,468,229. 8 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, 621, 4: 1132. 1949.

A block of cheese may be sliced in thin layers by this device which consists of a holder in which

the cheese is placed and a wire cutter which is pulled horizontally through the cheese. The cutter is positioned by a tortuous slot at both ends of the holder, the cutter being moved along said slot from one level to another to produce uniform slices.

R. Whitaker

Also see abs. no. 513, 520.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

509. Method of making low-ash crude lactose. E. F. ALMY and M. E. HULL. (Assigned to M & R Dietetic Laboratories, Inc.) U. S. Patent 2,467,453. 4 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, 621, 3: 781. 1949.

To sweet whey having a pH of 5.4 or higher is added sodium tetraphosphate in an amount sufficient to prevent heat coagulation and hydration of the protein. The whey then is condensed to a total solids content suitable for lactose crystallization.

R. Whitaker

510. The manufacture of cultured buttermilk from non-fat dry milk solids. R. N. COSTILON, M. L. SPECK and W. M. ROBERTS, N. Carolina State College, Raleigh. Milk Plant Monthly, 38, 4: 36-41. Apr., 1949.

Cultured buttermilk of good quality may be prepared from reconstituted non-fat dry milk solids. The suggested procedure for its manufacture involves pasteurization at 180° F. for 30 min., which sterilizes the reconstituted milk solids, yet does not impart a pronounced cooked flavor to the finished product. A slight preference exists for a 10% total solids content, since cultured buttermilk containing 8% solids was too thin, whereas the 11% product was too viscous. A resulting acidity, calculated as lactic acid, of 0.85 to 0.90% was found to be ideal from the consumer viewpoint. Following the above recommendations does not necessarily insure a premium product, since the flavor of the finished product cannot be better than the quality of the non-fat dry milk solids used in the preparation of the cultured buttermilk.

J. A. Meiser, Jr.

511. The manufacture, use and storage of dehydrated sweetened condensed milk. A. T. MUSSETT and W. H. MARTIN. Can. Dairy Ice Cream J., 28, 2: 68-74. Feb., 1949.

See abs. 88, p. A19.

512. Plastic cream—its production and uses. R. J. SPIERS. Can. Dairy Ice Cream J., 28, 4: 42, 46. Apr., 1949.

See abs. 87, p. A19.

Also see abs. no. 524, 525, 526, 545.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

513. The effect of penicillin on the acid-producing ability of starters. E. G. HOOD and H. K. KATZNELSON. Can. Dairy Ice Cream J., 28, 3: 32-33. Mar., 1949.

Where occasional mastitis-infected animals are treated with penicillin, the problem of inhibition of starter activity in the milk is not likely to present itself, owing to the dilution of the penicillin in the pooled milk supply. Its inhibitory effect can be a source of considerable concern to cheesemakers in areas where extensive use of the antibiotic is being made. It is suggested that the milk obtained from cows during the 3-d. penicillin treatment and for 1 d. thereafter, be used for purposes other than cheesemaking. A less costly method would be to inactivate the penicillin in the milk at the cheese factory using the enzyme penicillinase at the rate of 0.02 mg./100 ml. milk.

H. Pynson

514. Air express shipment of milk samples for bacteriological analysis. Anonymous. Milk Plant Monthly, 38, 4: 70-72. Apr., 1949.

Since many operators lack the ability to analyze microscopic counts correctly, a service whereby milk samples designated for bacteriological examination are flown by plane to a central laboratory was established. Shipping containers consist of an aluminum box filled with perforated airfoam rubber for receiving the sample bottles. Sterilized bottles containing formaldehyde are expressed to the dairy, the samples taken and returned by air express to the central laboratory. Since reports can be mailed out the following day, the time lag is not objectionable.

J. A. Meiser, Jr.

515. Molds in the dairy plant. S. G. KNIGHT, Univ. of Wisconsin, Madison. Milk Plant Monthly, 38, 4: 82-83. Apr., 1949.

Molds grow on a wide variety of materials under many different conditions. This fact necessitates the control of these simple plants if one wishes to produce products that do not possess a "musty" or rancid flavor. Although growth may occur anywhere in the plants, molds, like roaches, readily grow in inaccessible damp areas which are not cleaned properly.

Sanitation of wooden equipment involves constant and thorough cleaning followed by complete drying in dust-free air. Heavily infected equipment may be treated with chlorine solutions, hot

water or steam. Wrapping materials for dairy products may be freed of mold growth by soaking for 20 to 30 sec. in a 2 to 3% salt solution heated to 200° F. Also, 375 p.p.m. chlorine solutions and 10% calcium or sodium propionate solutions retard mold growth on wrapping materials.

Walls and shelves should be washed with alkaline phosphate solutions followed by a rinse with 1,000 p.p.m. chlorine solutions or 400 to 600 p.p.m. solutions of quaternary ammonium salts. Other methods for controlling mold growth on walls involve the use of a 4% borax solution, a 2% acetic acid solution, paints containing fungicides or ultraviolet lights. J. A. Meiser, Jr.

Also see abs. no. 502.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

516. Preliminary investigations of the determination of fully-saturated glycerides in New Zealand butterfat. I. TING, Fats Research Lab., Wellington, N. Z. New Zealand J. Sci. Technol., 29A, 5: 240-246. Feb., 1948.

Existing methods for determination of the fully-saturated glycerides in butterfat were found unsatisfactory. Higher yields of these glycerides were obtained by the author than by other workers. W. C. Frazier

517. De dispersiteit van karnemelkvat en het centrifugeren van karnemelk. (The distribution of buttermilk fat and the separation of buttermilk.) English summary. H. MULDER. Netherlands Milk Dairy J., 1, 1: 57-67. Jan., 1947.

From the data in the literature covered by this review, the fat in buttermilk can be divided into fat from the original milk fat globules, groups of globules and butter granules, colloidal fat and phospholipids. On comparing the methods of Rahn, Sandelin and Sirks for estimating the size distribution of fat globules in buttermilk, the method of Sirks appeared to be most accurate because it involves the measuring and counting of all fat globules. Some of the original milk fat globules and a relatively large quantity of colloidal fat remained in the buttermilk after centrifuging. W. M. Roberts

518. A general formula for the treatment of correction problems in the butyrometric determination of fat. TH. BROUWER. Netherlands Milk Dairy J., 1, 2: 98-109. Apr., 1947.

A formula was derived for correcting the butyrometric determination of fat so that it will be

comparable to the gravimetric method. The formula is:

$$C = B \left(\frac{100 SK}{Q} - 1 \right) + \frac{100 MK}{Q} + \frac{100 R}{Q}.$$

C = correction expressed in %; B = fat content read on the butyrometer; S = volume of one scale unit expressed in ml.; M = volume of the meniscus expressed in ml.; Q = weight of the quantity of substance taken in the operation, expressed in g.; K and R = constants. W. M. Roberts

519. The determination of diacetyl in butter. P. C. DEN HERDER. Netherlands Milk Dairy J., 1, 2: 110-113. Apr., 1947.

By using a specially constructed apparatus, the method of Prill and Hammer for the determination of diacetyl in butter was modified by substituting a CO₂-stream for the steam distillation. W. M. Roberts

520. Olika faktorers inverkan på löpekoaglets elastiska egenskaper. (Different factors affecting the elastic properties of rennet coagulum.) E. HANSSON, G. SJÖSTRÖM and E. G. SAMUELSSON, Kam. Bact. Inst., Alnarsinstitutets Mejeriavdelning. Svenska Mejeritidningen, 41, 5: 45-48. Jan., 1949.

The elasticity of the coagulum changes in direct proportion to the amount of rennet when this varies within the range of 3 to 20 parts of rennet to 10,000 parts of milk. Since the pH in milk diminishes with the addition of lactic acid, the elasticity of rennet increases in proportion to the change in the pH. The addition of CaCl₂ to milk causes an increase in the elasticity of the coagulum. This increase is greater than that brought about by the changes in the pH of milk. The elasticity increases constantly when the rennet temperature is between 25 and 30° C. Between 35 and 45° C. the increase in elasticity was not constant.

A "Konsistometer" is described. The elasticity is represented by the formula: $E = \frac{\varphi}{\delta} - 1$, where T = (in minutes) between the addition of rennet and measuring the elasticity; k = concentration of rennet in parts per 10,000 parts milk; φ = torsion angle measured in cm. on balance scales; and δ = opposite torsion angle for the cylinder. G. H. Wilster

521. Tocopherols (vitamin E) in milk: their chemical determination and occurrence in human milk. MARY LOUISE QUARF, Distillation Products, Inc., Rochester, N. Y. J. Biol. Chem., 169, 3: 513-514. Aug., 1947.

A method previously applied to the estimation of vitamin E in blood plasma has been adopted for milk. Because of the large proportion of low molecular weight triglycerides in butterfat, the molecular distillation technic is not applicable to milk. The method used employs solvent extraction and colorimetric assay. Values for winter cow's milk are quoted at from 17 to 30 γ /g. of butterfat, as compared with a group of summer milks with a mean value of 42 γ /g. of fat. Fifteen samples of human milk, collected within 1 wk. postpartum, gave values of from 76 to 1,800 γ /g. of fat, with 12 of the 15 samples exceeding 200 γ . Four composite samples of human milk, from 14 mothers in their first eighth month of lactation, showed tocopherol levels of from 37 to 58 γ /g. of fat.

A. O. Call

522. Instability of milk due to an increased activity of calcium ions. L. SEEKLES and W. TH. G. M. SMEETS. *Netherlands Milk Dairy J.*, 1, 1: 7-18. Jan., 1947.

Typical and non-typical cases of the "Utrecht abnormality (instability) of milk" are due to its increased calcium ion activity. The exact cause of this condition is not known but it may be due to mineral regulating processes in the animal body and factors which affect them. Typical cases often could be corrected successfully by oral or subcutaneous administration of sodium citrate. The instability of milk decreases as the pH is increased. Also, the condition was corrected by adding sodium citrate, a mixture of monopotassium and disodium phosphates, potassium oxalate or sodium flouride to the milk.

W. M. Roberts

523. The condition of casein in milk. P. VANDERBURG. *Netherlands Milk Dairy J.*, 1, 2: 69-77. Apr., 1947.

It was stated that casein occurs in milk as a salt of Ca; this salt keeps some colloidal Ca and PO_4 in suspension. Ca combines with the casein in an amount equivalent to the organic phosphate. From formaldehyde titration values obtained after heating milk, as well as after the addition of oxalate, it is concluded that the positive amino groups of lysine take part in the binding of the negative phosphate groups. This leaves only the negative carboxyl groups for the colloidal calcium.

W. M. Roberts

524. Method of treating casein products. J. G. WEELDENBURG. (Assigned to American Enka Corp.) U. S. Patent 2,468,531. 7 claims. Apr. 26, 1949. *Official Gaz. U. S. Pat. Office*, 621, 4: 1208. 1949.

Filaments of casein are made acid-resistant, the wet strength is increased and the swelling value reduced by reacting the casein with a solution of dimethylol and the monomethylol derivative of mono- and di-substituted phenols, then removing the excess solution, drying and heating.

R. Whitaker

525. Method of purifying casein. R. J. BLOCK and H. W. HOWARD. (Assigned to the Borden Co.) U. S. Patent 2,468,730. 6 claims. May 3, 1949. *Official Gaz. U. S. Pat. Office*, 622, 1: 125. 1949.

Casein in solution above pH 6 is coagulated by adjusting the pH to between 4 and 6 and, after filtering, is suspended in water. Sulphur dioxide gas is added to about pH 1.9 to produce a colloidal sol. The purified casein then is reprecipitated by adjusting the pH to between 4 and 6 and finally dried.

R. Whitaker

526. Method of producing egg substitutes. T. W. LINDEWALD and S. GRUBEN. (Assigned to Svenska Mjölprodukter Aktiebolag.) U. S. Patent 2,468,677. 6 claims. Apr. 26, 1949. *Official Gaz. U. S. Pat. Office*, 621, 4: 1246. 1949.

A substitute for egg albumin is made by drying skim milk treated with rennet at pH 6 to 7 in the presence of a substance capable of removing all of the dissociated calcium.

R. Whitaker

527. A study of whey proteins from the milk of various animals. H. F. DEUTSCH, Univ. of Wisconsin, Madison. *J. Biol. Chem.*, 169, 2: 437-448. July, 1947.

Milk serum proteins, obtained in most cases by removal of the casein from the skim milk by precipitation with rennin, were dialyzed, frozen and dried in vacuo. Electrophoretic mobility measurements, as well as sedimentation analyses (using an oil turbine Svedberg ultracentrifuge), then were made. The samples of milk tested were from the cow (no breed given), goat, pig (two breeds), human, sheep and horse. For each species samples included various days postpartum. Tables giving the results and also electrophoretic boundaries as well as sedimentation diagrams are included. Each species shows marked difference in the electrophoretic and sedimentation patterns of the whey proteins. The patterns change with the transition from colostrum to normal milk.

A. O. Call

528. Composition of Percheron mares' colostrum. A. D. HOLMES, A. F. SPELLMAN and R. T. WETHERBEE, Mass. Agr. Expt. Sta., Amherst. *J. Nutrition*, 37, 3: 385-392. Mar., 1949.

Thirty samples of colostrum were collected for the first 6 days of lactation from normal purebred Percheron mares. The initial samples of colostrum were not collected at the same interval following parturition. This possibly accounts for some of the wide variation of components in the first samples. Protein and magnesium content averaged higher in the first samples than in later samples. The amounts of ascorbic acid, phosphorus and potassium were lower in the initial samples than in later samples. Average values for individual mares ranged as follows: water, 86.3 to 88.1%; protein, 3.7 to 5.4%; ascorbic acid, 47 to 66 mg./l.; P, 75 to 86 mg./100 g.; K, 80 to 101 mg./100 g.; and Mg, 13 to 16 mg./100 g.

R. K. Waugh

529. Interaction of homologous alkyl sulfates with bovine serum albumin. F. KARUSH and M. SONNENBERG, N. Y. Univ. College of Medicine. J. Am. Chem. Soc., 71, 4: 1369-1376. Apr., 1949.

A colorimetric method for determining low concentrations ($10^{-5}M$) of homologous alkyl sulfates (dodecyl, decyl and octyl sulfates) was developed in order to study the reversible binding of these compounds by bovine serum albumin. Employing the method of equilibrium dialysis, experiments were conducted at room temperature ($25-28^{\circ}C.$) and at low temperature ($1-2^{\circ}C.$). The values of the thermodynamic functions ΔF° , ΔH° and ΔS° , for the binding process have been calculated. The binding of the alkyl sulfates is considered to be largely or wholly an entropy effect. However, no decision can be made as to the extent to which these changes are associated with the release of water molecules bound to protein and anion and/or structural changes of the protein. The binding of organic ions to serum albumin probably involves the electrostatic interaction between a positively charged group of the protein and the negative group of the anion. All of the positive groups on the protein are not available for binding. Results of this study show that only 14 groups are involved in binding, although the analytical data for bovine serum albumin indicate that at pH 6.1 the arginine and lysine residues alone account for 84 such groups. Considering the possibility that a variation of the intrinsic association constants of the binding sites would account for the shape of the binding curves observed, a quantitative formulation has been proposed. The new theory describes the heterogeneity associated with differences among the sites on the same molecule.

H. J. Peppler

530. Studies on proteins from bovine colostrum. I. Electrophoretic studies on the blood serum proteins of colostrum-free calves and of calves fed

colostrum at various ages. R. G. HANSEN and P. H. PHILLIPS, Univ. of Wisconsin, Madison. J. Biol. Chem., 171, 1: 223-227. Nov., 1947.

The blood serum proteins of calves were measured electrophoretically before and after they had ingested colostrum and at various ages. When colostrum was fed within 24 hr. after birth there was a marked increase in the γ -globulin in the blood serum, but when colostrum was fed after the calves were 24 hr. old there was no such increase of this blood serum fraction. The same effect and the same limitation as to age were found when a water-soluble globulin was isolated from colostrum, added to warm skim milk and fed the calves. When calves were not given colostrum within 24 hr. after birth, it required about 8 wk. for their serum blood protein fractions to approach normal values.

A. O. Call

531. Studies on proteins from bovine colostrum. II. Some amino acid analyses of a purified colostrum pseudo-globulin. R. G. HANSEN, R. L. POTTER, and P. H. PHILLIPS, Univ. of Wisconsin, Madison. J. Biol. Chem., 171, 1: 229-232. Nov., 1947.

A water-soluble fraction of bovine colostrum referred to as pseudoglobulin was isolated and purified by repeated precipitation and solubilization. Sedimentation analyses, using a Svedberg oil turbine ultracentrifuge, and also electrophoretic analyses indicated that the fraction was homogeneous. Determinations were made for fourteen different amino acids and the percentages are given in a table compared with reported values for the same amino acids as found in human γ -globulin. In general, the colostrum globulin is similar in amino acid content to the human γ -globulin except that it shows a somewhat higher level of proline and isoleucine and the lysine value is somewhat lower than that reported for human γ -globulin.

A. O. Call

532. Biophysical studies of blood plasma proteins. IX. Separation and properties of the immune globulins of the sera of hyperimmunized cows. E. L. HESS and H. F. DEUTSCH, Univ. of Wisconsin. J. Am. Chem. Soc., 71, 4: 1376-1381. Apr., 1949.

Ethanol fractionation has been applied to the recovery of the antibody-enriched fractions of hyperimmune sera prepared by inoculating 2 Holstein cows with Newcastle virus in whole egg embryo and with viable *Brucella abortus* suspensions at 4-d. intervals over a 3-mo. period. *B. abortus* agglutinins and bactericidins and Newcastle virus hemagglutination inhibiting and neutralizing antibodies were assayed. Increases in the

content of the serum γ -globulins were followed by electrophoretic analysis. The major portion of the antibodies was precipitated from the diluted serum at pH 7.6 and 18% ethanol, yielding more than 90% of the γ -globulins present in the original serum. Antibodies of *B. abortus* differed in solubility; the bactericidins were comparatively more soluble than the agglutinins. Subfractionation of the γ -globulins revealed the presence of the neutralizing antibodies for Newcastle virus in both the γ_1 - and γ_2 -globulin fractions; the hemagglutination inhibiting antibodies appeared to remain largely in the γ_1 -globulin fraction. The concentration of neutralizing antibodies for Newcastle virus and bactericidins and agglutinins for *B. abortus* was distinctly lower in the γ_2 -globulin fraction. Electrophoretic spreading experiments and solubility studies indicated that the γ -globulin fractions are electrically inhomogeneous. Their sedimentation behavior, however, revealed an essentially monodisperse system. H. J. Peppler

Also see abs. no. 498, 506, 538.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

533. Butter manufacturing process. H. C. HORNEMAN, R. V. HUSSONG, S. N. QUAM and B. W. HAMMER. (Assigned to Sugar Creek Creamery Co. and Cherry Burrell Corp.) U. S. Patent 2,466,894. 16 claims. Apr. 12, 1949. Official Gaz. U. S. Pat. Office, 621, 2: 494. 1949.

Butter is produced continuously from cream through the following series of steps: preheating, filtering, pasteurizing by a steam-vacuum treatment, centrifuging to produce milk fat, blending the fat with water and salt in a mixing chamber, cooling to stabilize the emulsion and finally working into butter. R. Whitaker

534. Butter manufacturing means and method. H. C. HORNEMAN, R. V. HUSSONG, S. N. QUAM, and B. W. HAMMER. (Assigned to Sugar Creek Creamery Co. and Cherry Burrell Corp.) U. S. Patent 2,466,895. 18 claims. April 12, 1949. Official Gaz. U. S. Pat. Office, 621, 2: 494. 1949.

Essentially the same as abs. no. 533 covering patent 2,466,894 with details given for controlling the crystallization of the blended milk fat, water and salt to yield a butter of good texture by means of a freezer-type cooler and a tubular texture developer equipped with restricted orifices. R. Whitaker

535. Dairy Process. H. C. HORNEMAN, R. V. HUSSONG, S. N. QUAM and B. W. HAMMER. (Assigned to Sugar Creek Creamery Co. and Cherry Burrell Corp.) U. S. Patent 2,466,896. 12 claims. Apr. 12, 1949. Official Gaz. U. S. Pat. Office, 621, 2: 494. 1949.

A dairy product, having many of the characteristics of butter, is produced by the same sequence of steps as described in abstract no. 533 covering U. S. patent 2,466,894. R. Whitaker

536. Practical ammonia refrigeration for ice cream and milk plants. CLYDE H. MINSTER, Greenbrier Dairy Products Co., Beckley, W. Va. Ice Cream Rev., 32, 9: 44, 94, 96, 98, 100, 102, 104, 106. Apr., 1949.

In determining the refrigeration load, the author suggests the use of a flow sheet depicting each operation involved in processing of the various products of the plant. The diagrammatic flow sheet presented with the article should serve as a useful guide in the preparation of such a chart.

The refrigeration load in the typical milk plant is mostly the cooling of products and product containers. Sweet water cooling with an ice bank hold-over system is suggested as an economical method for cooling raw milk since this system permits storage of refrigeration in the form of ice which in turn will reduce the size of compressor required. Further economies can be effected in the refrigeration load if the raw milk which is to be separated can be by-passed around the raw milk cooler directly to the preheater. The full use of regeneration whenever possible in cooling milk following pasteurization will conserve both heat and refrigeration. Milk or milk products placed in warm containers frequently will show an appreciable temperature rise which is detrimental to its quality. The cooling of the containers constitutes a portion of the refrigeration load which frequently has been overlooked. To date no satisfactory method has been devised for adequate precooling of milk bottles prior to filling. The temperature of storage rooms for bottled milk should not be permitted to rise above 33° F. In addition to temperature control, good air circulation is essential in the storage room.

The refrigeration load for the ice cream plant involves: (a) cooling and storage of mix, (b) partial freezing in the freezer, (c) hardening and storage of the ice cream and (d) freezing of novelties with low temperature brine. Direct expansion ammonia systems are best adapted for the freezing and hardening of ice cream. The freezing of novelties is carried out with a low temperature brine which in turn is cooled to the desired temperature with ammonia coils. Mix cooling can be carried out with either sweet water, brine or direct expansion. W. J. Caulfield

537. Ammonia equipment in ice cream plants. V. C. PATTERSON. Can. Dairy Ice Cream J., 28, 4: 72-76. Apr., 1949.

See abs. 454, p. A94.

Also see abs. no. 554, 555, 573, 574, 575.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

538. De bepaling van het vetgehalte en de invloed van systematische fouten op de verliescijfers der vetbalans. (The estimation of the fat content and the influence of systematic errors on the figures of loss in the balance-sheet for fat.) English summary. A. F. TAMSMA, H. J. J. JANSSEN, and S. A. H. PASSENIER. Netherlands Milk Dairy J., 1, 2: 78-97. Apr., 1947.

In checking the amount of butterfat lost in dairy plant operations, the reliability of the figures has been found to depend to some extent upon the methods used for routine butterfat determinations. Analytical errors accounted for about 2% fat loss when the Gerber test was used as the basic method. Either a test must be found which is sufficiently accurate and will give a fat loss of 0% in the ideal case or a correction factor should be used. The Weibull method is suggested as being very satisfactory. If the Gerber test is used, the correction factor would have to amount to 2%, i.e., the Gerber fat content must be multiplied by 0.98. W. M. Roberts

539. Modern trends in self-service and package merchandising of ice cream. H. H. ROBBINS, Paraffined Carton Research Council. Ice Cream Trade J., 45, 4: 54, 55, 116-118. Apr., 1949.

Merchandising fundamentals that will make for increased sales include quality of product, proper packaging, display and merchandising and advertising. The tremendous growth of self-service type of merchandising in food stores has resulted in the necessity for placing more emphasis on packages and impressing brand names on the consumer's mind.

From 1938 to 1947 groceries handling ice cream have increased from 22 to 68%. New household refrigerators with low-temperature storage compartments provide ample space for storage of packaged ice cream. Low-temperature storage boxes for home and farm use make ice cream available to many people who have had it only occasionally. New automatic packaging machinery has reduced packaging costs. With facilities for storage of ice cream in the home, consumers are purchasing in larger amounts and new outlets make ice cream more readily available. Dealers are becoming more interested in selling packaged ice cream because it has eliminated dipping losses. Improved quality and the introduction of the higher quality, low-overrun ice cream has increased home consumption. Open display cabinets and mobile cabinets have increased sales of packaged ice cream. Aggressive

merchandising by drug stores is increasing the sale of ice cream not only at the fountain but also for carry-home use. W. H. Martin

540. Carnation hits hard for more sales. ANONYMOUS. Ice Cream Field, 53, 3: 46-48, 50-51. Mar., 1949.

This is a condensation of the Feb. issue of *The Mixer*, a monthly publication of the Carnation Co., Los Angeles, Calif. Charts and statistics are given to stress the advantages of ice cream in drug stores and markets. The Rexall survey (1929) is quoted as saying that "For every \$1.00 the fountain brings in, the store volume in other departments increases \$0.93." The advantages of rapid turn-over are emphasized by charts. Drug stores are urged to consider shorter gross profit on packaged ice cream in order to increase sales volume. It is suggested that a "take home" cabinet, separate from the fountain, serves as an additional display; furthermore, this method of selling ice cream is less costly than selling at the fountain. The Hotel Gazette (1943) is quoted as finding that America's favorite dish is ice cream. W. C. Cole

541. How to increase per capita sales. M. L. FINNEBURCH, Liquid Carbonic Corp., Chicago, Ill. Ice Cream Field, 53, 3: 44, 60, 62, 64, 65. Mar., 1949.

To emphasize the importance of retail outlets for ice cream, the following statistics are given: Forty million Americans eat away from home every day. In drug stores 43% of the multiple sales start at the soda fountain, 25% of the average store volume comes from the soda fountain, 31% of the store gross profits come from the soda fountain and, on the average, the soda fountain shows a net profit of 14%. The author emphasizes the increased profits resulting from increased sales. He classes the soda fountain as a "food and refreshment factory", claiming it is the "Personality" department and, because of this, has a distinct advantage from the sales point of view. W. C. Cole

542. "One-stop-saver" plan. V. M. RABUFFO. Ice Cream Trade J., 45, 4: 44-46, 108-110. Apr., 1949.

Stores using the plan must phone orders to the company during the day prior to delivery; the minimum order is for 5 gal. of ice cream or 12 dozen novelties at one delivery and the store personnel must take the ice cream from the truck at the curb and place it in the cabinet in the store. The price is based on the quantity delivered, ranging from \$1.46 per gal. for 5 to 9 gal., to \$1.37 per gal. for 50 gal. or more at one delivery. W. H. Martin

543. Prices drop. Ice Cream Trade J., 45, 4: 40-42, 97-104. Apr., 1949.

Wholesale prices of ice cream have been reduced from 8 to 25 cents per gal. in most of the major markets throughout the United States during the past 2 mo. These reductions have been possible as the result of lower prices for milk and milk products used in the manufacture of ice cream. It is the hope of the industry leaders that these price reductions will be passed on from the dealers to the consumers and that increased sales will result.

W. H. Martin

544. A double incentive plan. T. KNIGHT. Milk Plant Monthly, 38, 4: 50-51. Apr., 1949.

Providing a routeman's outstanding bills are lower than in the preceding month, he receives \$1.50 for each new quart of business secured. This bonus is reduced to \$1.00 if his outstanding bills exceed those of the preceding month. Accurate recording and prompt posting of this collection data by the sales manager make this plan an effective method of promoting sales and speeding up collections.

J. A. Mciser, Jr.

545. Cost problems of the dry milk industry. D. BUTZ and E. F. KOLLER, Univ. of Minnesota, St. Paul. Natl. Butter Cheese J., 40, 5: 36-37, 60. May, 1949.

The costs of manufacturing 1 lb. of milk powder by the spray and roller methods in 1947 were 4.4 and 3.7 cents, respectively. Labor, packaging supplies and fuel compose 0.75 of the manufacturing cost. Manufacturing costs were 1 cent/lb. lower in spray-drying plants producing over 6 million lb. of powder annually than in plants producing less than this amount. Indications for 1948 show that labor and fuel costs increased. The estimated average manufacturing cost in 1948 will be 0.5 cent/lb. higher than in 1947.

Seasonality of products is a big factor in powder manufacturing costs. Labor and equipment are designed to handle milk flow during peak periods, resulting in less efficient use of labor and equipment at other times of the year. Diversity of plant operations may be an answer to this problem. The introduction of more labor-saving equipment and methods would cut manufacturing costs. More effective utilization of fuel should be studied.

H. E. Calbert

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

546. Effect of vitamin A supplements upon the state of vitamin A in blood serum of the dairy cow and in blood serum and liver of its neonatal calf. D. B. PARRISH, G. H. WISE, and J. S.

HUGHES, Kansas Agr. Expt. Sta., Manhattan. J. Biol. Chem., 172, 2: 355-365. Feb., 1948.

Sixteen pregnant cows (5 Holstein, 3 Ayrshire, 4 Guernsey and 4 Jersey) were divided into two groups about 4 wk. before parturition, one group being given supplements of vitamin A in the form of the alcohol and the other vitamin A in the form of the ester. Before this supplementation their blood serum averaged 34 γ total vitamin A/100 ml., of which 10% was in the form of the ester. Following daily oral doses of 500,000 I. U., and later 1 million, of both the alcohol and the ester, the total vitamin A level of each group increased to an average of 45 γ /100 ml. of serum, of which 24% was in the form of the ester. At parturition the supplementations were discontinued, resulting in a decrease to 28 γ /100 ml., of which 9% was in the ester form. There was no significant difference between the groups receiving the two different forms of vitamin A.

Fifteen newborn male calves (6 Ayrshire, 4 Holstein, 3 Guernsey and 2 Jersey), of which 6 were from the dams on the experiment above, comprised a group used to determine the level of vitamin A in their blood serum and livers. With one exception, they were 4 d. or less of age. The blood serum of 10 of the calves showed vitamin A values of from 5 to 22 γ /100 ml. Of the total vitamin A in their blood serum, 2 calves showed more than 60% of it to be in the form of the ester and the other 8 had a fourth or less of their serum vitamin A in the ester form. Fourteen of the group were sacrificed for vitamin A assays of their livers. Although the total vitamin A of their livers varied widely, depending upon the supplement of their dams, in all cases more than 70% was present in the ester form.

A. O. Call

547. The lactation response as limited by feeds produced under two systems of soil fertilization. K. A. KENDALL, W. B. NEVENS and O. R. OVERMAN, Univ. of Illinois, Urbana. J. Nutrition, 36, 5: 625-637. Nov., 1948.

The effect of fertilizer treatment of soils on the nutritive value of rations consisting of lespedeza hay and wheat grain and of soybean hay and wheat grain were appraised through reproduction and lactation responses of rabbits. All plots on which crops were grown for this study received 4 tons of raw rock phosphate, 2.25 tons of kainit and 1,000 lb. of muriate of potash over a period of 12 yr. In addition, one series of plots was treated with 12 tons of limestone over this same period.

The lespedeza and wheat ration from the soil fertilized with PK + Ca appeared to be slightly more palatable than the same ration from soil

which did not receive Ca. Rabbits receiving the rations from soil receiving only PK averaged 6.43 young per litter while those fed rations from soils receiving PK + Ca averaged 7.75 young. Average daily weights, body length and gastrointestinal contents, and deposition of dry matter, total nitrogen and ash were greater for young fed on milk from mothers which received rations from soils fertilized with PK + Ca than those fed on milk from mothers which received rations from soils treated only with PK. R. K. Waugh

548. The placental and mammary transfer of tocopherols (vitamin E) in sheep, goats and swine. F. WHITING and J. K. LOOSLI, Cornell Univ., Ithaca. *J. Nutrition*, **36**, 6: 721-726. Dec., 1948.

The effects of supplementing prepartum rations with tocopherols on the tocopherol content in livers and blood plasma of newborn, and on the amount of tocopherol in colostrum were studied. Half the animals received rations supplemented with 80 mg. of γ -tocopherol per 100 lb. of body weight daily until parturition. The other half served as controls. Samples from the newborn were taken before they had received colostrum and the colostrum was sampled before the young were suckled.

The supplementation of the mothers' prepartum ration slightly increased the tocopherol content of livers of new born. This increase was not statistically significant. The tocopherol content of blood plasma of lambs and kids was increased significantly by the prepartum supplementation, but no increase was observed with newborn pigs. Supplementation resulted in a two-fold increase in tocopherol content of colostrum in all species. Colostrum contained 3 to 4 times as much tocopherol as milk from the same species 4 d. later. R. K. Waugh

549. The effect of mixed tocopherols on milk and butterfat production of the dairy cow. P. H. PHILLIPS, J. KASTELIC and E. B. HART, Univ. of Wisconsin, Madison. *J. Nutrition*, **36**, 6: 695-701. Dec., 1948.

A farm herd of Holstein cattle was used to study the effect upon per cent butterfat, total fat and milk production of supplementing rations with 1 g. of mixed tocopherols per cow daily. H.I.R. monthly tests and milk records were used for the production data. In trial I 7 cows were used as controls and 8 other cows received daily 21.3 g. of a preparation which furnished 1 g. of mixed tocopherols. The tocopherols were added to the evening feed. In trial II 7 cows which had received tocopherols and 4 cows which had served as controls in trial I were fed a grain ration with which the tocopherols had been mixed at the time

of grinding the grain. Neither method of feeding the mixed tocopherols had any effect on per cent butterfat, total butterfat or milk production.

R. K. Waugh

Also see abs. no. 530.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

550. The amino acid composition of bovine semen. B. C. RAY SARKAR, R. W. LUECKE, and C. W. DUNCAN, Michigan State College, East Lansing. *J. Biol. Chem.*, **171**, 2: 463-465. Dec., 1947.

A composite of 149 semen samples from 40 different bulls representing Holstein, Guernsey and Jersey breeds was separated into sperm and seminal plasma by centrifugation. Each fraction then was dried and analyzed microbiologically for eleven different amino acids. The results are presented in a table. The amino acid compositions of sperm and seminal plasma are quite similar, except that arginine and leucine are higher in sperm while tryptophan is higher in the plasma. Nitrogen values of 17.61 and 12.05%, respectively, for the sperm and seminal plasma on a moisture-, fat- and ash-free basis are reported. A. O. Call

Also see abs. no. 488, 552.

HERD MANAGEMENT

II. A. HERMAN, SECTION EDITOR

551. Breed type and production records in Jersey cattle in New Zealand. A. H. WARD and O. M. CASTLE, New Zealand Dairy Board. *New Zealand J. Sci. Technol.*, **29A**, 4: 175-183. Dec., 1947.

Records on cows are classified according to New Zealand standards into: very highly commended, highly commended, commended and commended for superior progeny.

W. C. Frazier

552. Average length of gestation period in dairy cattle in New Zealand. A. H. WARD and O. M. CASTLE, New Zealand Dairy Board. *New Zealand J. Sci. Technol.*, **29A**, 4: 171-173. Dec., 1947.

The length of the gestation period for 2,255 cows conceiving within a 6-mo. period was summarized. The average length of gestation period for single calves was 283 ± 11.3 d. Some 85 cows had a period of less than 267 d and 87 a period of greater than 299 d. The gestation period for single births is approximately 4 d. longer than for twin births. Significant differences between

average gestation periods in different sections of New Zealand were observed W. C. Frazier

553. Method of making milker nipples. C. A. THOMAS. (Assigned to Babson Bros. Co.) U. S. Patent 2,463,920. 7 claims. Mar. 8, 1949. Official Gaz. U. S. Pat. Office, 620, 2: 582. 1949.

On the type of milker which has the milk-collecting vessel directly beneath the cow, the vacuum automatically is cut off when the teat cup drops off. This is accomplished by having the flexible hose to the teat cup slip over a rigid tube cut at an angle of about 22.5° with the flat side facing upward. When the teat cup hangs down, the wall of the flexible tube covers the flat end of the rigid tube, thus preventing loss of vacuum.

R. Whitaker

554. Automatic shut-off mechanism for milking machines. M. K. EADES. U. S. Patent 2,466,841. 8 claims. Apr. 12, 1949. Official Gaz. U. S. Pat. Office, 621, 2: 479. 1949.

A float-operated valve automatically stops the milking machine from operating when the flow of milk ceases.

R. Whitaker

555. Milking apparatus. A. C. WEIBY. (Assigned to Solar Corp.) U. S. Patent 2,467,512. 2 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, 621, 3: 796. 1949.

To provide the pulsating vacuum required for a milking machine, a rotor periodically connects the pulsing line with the suction source and periodically connects the pulsing line with the atmosphere.

R. Whitaker

556. Strip cup. C. A. THOMAS. (Assigned to Babson Bros. Co.) U. S. Patent 2,467,949. 3 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, 621, 3: 908. 1949.

This strip cup has a cover in the shape of a shallow funnel. Milk from the udder is directed on the surface, where it spreads out to form a film and then drains down into the cup below through the center opening.

R. Whitaker

Also see abs. no. 487.

ICE CREAM

C. D. DAHLE, SECTION EDITOR

557. Defects in ice cream. C. D. DAHLE, Pennsylvania State College. Ice Cream Trade J., 45, 4: 48, 49, 110, 111. Apr., 1949.

The causes and remedy for common flavor defects are discussed. These include old material, unclean, cooked, neutralizer, salty, sour, oxidized and rancid flavors. Body defects discussed in-

clude sogginess, fluffy, gummy and crumbly. Texture defects include coarse, buttery, sandy and poor melt-down characteristics.

Shrinkage is discussed in detail, including the effects of continuous freezers, dry ice, paper containers, storage temperatures, overrun, types of sweeteners, stabilizers, emulsifying agents, dryness of ice cream, free-fatty acids, protein stability, composition of the mix, types of dairy products and seasons of the year. Shrinkage theoretically is attributed to the escape of air from the air cells by diffusion or collapse of the air cell wall due to pressure from within or without the cell. Wall strength is important in retaining air within the cell. Shrinkage may be attributed to some of the fundamental factors which have a definite effect on the behavior of milk solids during certain seasons of the year. W. H. Martin

558. Significance and control of coliform in ice cream making. G. W. SHADWICK. Can. Dairy Ice Cream J., 28, 3: 74-78. Mar., 1949.

See abs. 441, p. A92.

559. Production and pasteurization of ice cream mix by H.T.S.T. method. C. M. MINTHORN. Can. Dairy Ice Cream J., 28, 2: 76-78, 90. Feb., 1949.

See abs. no. 466, p. A98

560. Controlling viscosity of chocolate ice cream mixes. C. D. DAHLE, W. R. DAVEY and W. D. SWOPE. Can. Dairy Ice Cream J., 28, 4: 58-64. Apr., 1949.

See abs. 471, p. A99.

561. Ice cream cup. A. A. HEYMAN. (Assigned to Maryland Baking Co.) U. S. Patent 2,462,497. 2 claims. Feb. 22, 1949. Official Gaz. U. S. Pat. Office 619, 4: 1074. 1949.

A flat-bottomed ice cream cone is described which has a series of protruding arms of pastry around the inner periphery and also upward from the bottom which impart the following features to the cone: the portion of ice cream is supported on top of the cone, suggesting overfilling and preventing accidental spilling, the cups may be nested without breaking the nesting rings and the ice cream may be nearly all consumed before the cup need be eaten.

R. Whitaker

562. Prospectus for packages. W. D. KELLOGG, Container Corp. of America, Chicago, Ill. Ice Cream Field, 53, 3: 75. Mar., 1949.

Packaged ice cream provides a means whereby the industry may attain its announced goal of a billion gal. of ice cream per yr. Education, up-

grading, reduction in distribution costs and better merchandising are steps listed as necessary if this goal is to be attained. Dispensing ice cream in packages is more sanitary and more economical and permits better merchandising through more attractive designs; it is necessary in self-service stores.

Making a factory-filled package comparable in quality to "hand-dipped" ice cream is essential if packaged ice cream is to be accepted universally. The need of proper carry-out insulated bags as well as adequate home refrigerator storage are stressed as a means of convincing consumers they should maintain a supply of ice cream in their homes. W. C. Cole.

Also see abs. no. 536, 537, 539, 540, 541, 542, 543.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

563. One-piece paper container. J. NORDEN. U. S. Patent 2,468,306. 4 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, **621**, 4: 1152. 1949.

A square-shaped paper bottle suitable for holding liquids like milk is constructed from one long strip of paper which is wound spiral-like with the edges overlapping and terminating at the mouth or open end. R. Whitaker

564. Milk can inverting fork truck. T. A. GLEASON. U. S. Patent 2,468,326. 5 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, **621**, 4: 1157. 1949

This attachment for the front of a small truck consists of fork-shaped arms which are employed to lift cans of milk upward and then inverting same over a tank or other receptacle. R. Whitaker

565. Fat-free milk. K. G. WECKEL. Can. Dairy Ice Cream J., **28**, 3: 80, 86, 101. Mar., 1949.

See abs. 391, p. A81.

566. Problems in the operation of city milk plants. L. W. HOYT. Can. Dairy Ice Cream J., **28**, 3: 57, 58, 100. Mar., 1949.

See abs. 235, p. A52.

Also see abs. no. 522, 523, 536, 544, 569, 571, 572.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

567. Reproduction and lactation studies with rats fed natural and purified rations. G. M. MARUYAMA and P. H. PHILLIPS, Univ. of Wisconsin, Madison. J. Nutrition, **36**, 5: 613-623. Nov., 1948.

The adequacy of a ration consisting of corn, soybean oil meal, dehydrated alfalfa leaf meal, minerals and supplements of most of the known vitamins was tested for reproduction and lactation in rats in experiment I. The rats were fed the basal ration for 3 wk. prior to mating. The importance of standardizing these and other factors for reproduction and lactation studies is emphasized. Supplementing the diet with 0.5% L(+)-lysine and 0.3% DL-methionine improved reproduction and lactation, the percentage of rats born that were weaned increasing from 46 to 72%. When folic acid was omitted from the basal ration only 34% of the young born were weaned.

In experiment II a purified ration consisting of sucrose, casein, corn oil, minerals and vitamins was employed, in addition to the basal ration of natural materials used in experiment I. In this trial omitting the folic acid from the diets reduced the weaning percentage from 29 to 0 for the basal made of natural materials and from 59 to 10 for the purified.

Lysine, methionine and folic acid apparently are essential for good reproduction and lactation in the female rat. R. K. Waugh

Also see abs. no. 531, 547, 548.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

568. Research on a growth-promoting factor in summer butter. A. KENTIE. Netherlands Milk Dairy J., **1**, 2: 118-127. Apr., 1947.

The fatty acids of summer butter contain a growth-promoting factor for rats. The factor can be adsorbed on Fuller's earth but loses its growth-promoting properties upon complete hydrogenation. W. M. Roberts

569. De voedingswaarde van melk. (Nutritive value of milk.) English summary. CHR. ENGEL. Netherlands Milk Dairy J., **1**, 1: 43-56. Jan., 1947.

Data have been collected from recent literature about the amino acids, fatty acids, vitamins and mineral components of cow's milk and human milk. W. M. Roberts

Also see abs. no. 521.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

570. Residual arsenic and strychnine in the tissues of drug-treated cattle. W. E. HAM, E. A.

KLINE and M. E. ENSMINGER, State College of Washington, Pullman. *Am. J. Vet. Research*, 10, 35: 150-153. Apr., 1949.

Arsenic trioxide and nux vomica were fed in the grain ration to groups of cattle for 120 d. in 1946 and 201 d. in 1947. Average daily intakes were 0.357 g. As_2O_3 and 5.69 g. nux vomica. Biopsy and slaughter tissues analyzed at the end of the trial showed levels of arsenic approaching or exceeding the maximum allowed by Pure Food and Drug regulations. Tissues analyzed after depletion periods of 20 and 41 d. contained about the same amount of arsenic as found in untreated control animals. Strychnine was not found in the tissues or organs at any of the sampling periods. Effects of the drugs upon blood glucose, ascorbic acid, carotene, vitamin A, calcium, phosphate and cell count were not significant.

E. W. Swanson

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

571. **The fieldman's role in producing quality milk.** II. A. BOLAND, Galliker Dairy Co., Johnstown, Pa. *Milk Plant Monthly*, 38, 4: 42-45. Apr., 1949.

Analysis of laboratory reports and personal contact with producers and fieldmen shows the major causes of bacteriological problems in relations to the dairy farm to be: (a) improper care of mechanical coolers, (b) improper care of milking machines, (c) confusion as to the proper use of washing compounds and chemical sterilizers, (d) failure on the part of fieldmen to locate the cause of trouble and (e) fieldmen improperly equipped to do the job.

Fieldmen must locate trouble spots quickly and then insure that the producer possesses the necessary tools and is using them properly and effectively in erradicating this defect. Also, the fieldmen should possess tools which allow him to perform his duties with a minimum of guesswork. These tools properly used and built into a compact kit will take much out of the guesswork in maintaining clean milk production.

J. A. Meiser, Jr.

572. **Cleaner dairy equipment.** M. C. JAMIESON and W. G. McLEOD. *Can. Dairy Ice Cream J.*, 28, 4: 27-31. Apr., 1949.

Results of a new type of sanitary program conducted among milk producers in Manitoba are discussed. The "Seeing is Believing" test (the Jamieson Kit) is applicable for producers as well as manufacturers. It provides a measure of sanitation on the farm and in the plant. The program of education conducted in this study has

proven that producer education in sanitation is needed and appreciated and that improvement is possible.

H. Pyenson

573. **Can washers.** H. P. FAUST. *Can. Dairy Ice Cream J.*, 28, 4: 78-80. Apr., 1949.

A rotary can washer usually answers the cleaning problem where a rate of four cans a minute is adequate and the total number of cans is not large. The increase in labor cost has made the straightway can washers more common. The rate of washing cans should be integrated with the weighing, cooling and storage of milk. Other questions to be considered are: (a) adequacy of water supply, (b) hardness of the water, (c) boiler capacity for peak loads, (d) adequate size of water and steam mains to prevent pressure drops when other equipment is operated and (e) proper electrical lines and voltage maintenance. The prewash rinse should be provided with means of raising the water temperature, particularly in winter; in the washing positions a large volume of water at relative low pressures is necessary; only a small rinse pump should be necessary; a small volume of water at a relatively high temperature is desirable in the sterile rinse position; and a limited volume of steam is needed in the steam sterilization position.

H. Pyenson

574. **Can-washing operation and maintenance.** C. A. ABELE. *Can. Dairy Ice Cream J.*, 28, 4: 82-84, 90. Apr., 1949.

Provision of a mechanically efficient can-washer, charged with an effective washing compound, does not assume the delivery of completely cleaned milk cans at the discharge end of the washer. The third factor essential to satisfactory can-washing results is intelligent operation. The most effective method of determining the effectiveness of the nozzle jets is to run a cut-out can through the washer, with the side panels removed, before the start or after the clean-up following each day's run. The maintenance of wash solution concentration is very important.

H. Pyenson

575. **How polyphosphates improve can washing.** A. H. RAZEE. *Can. Dairy Ice Cream J.*, 28, 4: 86-90. Apr., 1949.

Polyphosphates soften the hardest water, thereby preventing the precipitation of detergent materials. They also eliminate the formation of film and scale on the equipment and on the cans being washed. In alkaline solution, the polyphosphates are specific solvents for denatured proteins, such as are found in milkstone. These polyphosphates can be used to treat rinse water so that even upon dilution, the residual alkali

will not precipitate. In the field of dairy sanitation, the polyphosphates have been acclaimed as one of the most significant developments in detergency.

H. Pyenson

576. Chemical sterilizers in the dairy industry.
C. K. JOHNS. *Can. Dairy Ice Cream J.*, **28**, 3:
29-31. Mar., 1949.

The following suggestions are given in regard to the use of chemical sterilizers: (a) have equip-

ment surfaces adequately cleaned, (b) prepare sterilizing solution according to directions, preferably in hot water, (c) hypochlorite is best applied immediately before using the equipment (quaternaries may be used following wash-up), (d) hypochlorite solutions above 5% strength are unstable and lose available chlorine and (e) crystalline hypochlorite should be kept tightly covered, as exposure to the air causes a decrease in strength.

H. Pyenson

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEW

577. Condensed milk and milk powder. 7th ed. O. F. HUNZIKER. 583 pp. Published by the author, La Grange, Ill. 1949.

This new edition follows the pattern of the preceding edition, with the incorporation of an appreciable amount of new material. The cuts of equipment have been brought up-to-date in many instances and some of the newer methods of processing which have been used in European countries are described and illustrated. The chapter on "Definitions, Standards and Import Tariffs" has been expanded to incorporate recent material. The increased number of pages is due, in part, to less crowding on the pages and more readable type. The appearance of the new edition after only 3 years is evidence of the desire of the author to keep this standard textbook abreast of the new developments in the field.

F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

578. Effect of infused streptomycin in the mammary gland. C. R. SMITH, W. E. PETERSEN AND R. W. BROWN, Univ. of Minnesota, Minneapolis. Proc. Soc. Exptl. Biol. Med., **68**, 1: 216. May, 1948.

Five grade Holstein-Friesian cows and one grade Togenberg goat were employed. All animals were lactating normally and free of disease during the experiment. The cup-plate method of assay, as adapted to measurement of antibiotics in milk, was used for determining the concentration of streptomycin in the milk samples. Milk samples for assay purposes were obtained by milking out the quarter completely. Streptomycin could be detected in milk samples as long as 48 hr. after the infusion of 100,000 to 500,000 units/quarter and concentration did not fall below 20 units/ml. in any of the samples after a 24-hr. interval. Concentration was found to vary with dose, interval between infusion and sampl-

ing, and milk production. At no time was it possible to detect streptomycin in the blood. However, in both the cow and goat, significant amounts were found in urine samples as long as 27 hr. following infusion. The pH, chlorides, cell count and clinical inspection showed that streptomycin was relatively non-toxic when infused into the normal bovine mammary gland.

R. P. Reece

579. Comparison of New Jersey and Palestine strains of bovine leptospira. H. BERNKOPF AND R. B. LITTLE. Rockefeller Inst. Med. Research, Princeton, N. J. Proc. Soc. Exptl. Biol. Med., **69**, 3: 503-506. Dec., 1948.

A study was made of strains of leptospira recovered from an outbreak of leptospirosis among cattle in New Jersey and a strain isolated from an outbreak of the disease in Palestine. In agglutination and lysis tests on sera from recovered cattle as well as sera from immunized rabbits, all the New Jersey strains reacted alike, while the Palestine strain belonged to another serological group.

R. P. Reece

580. Brucellosis: Contralor sanitario de los alimentos. (Brucellosis: Sanitary Control of Foods.) E. PIERANGELI. Rev. asoc. argentina dietol., **5**, 50: 281-284. Oct., Nov., Dec., 1947.

Direct and indirect transmission of the disease is discussed. The author states that contraction of the disease from the bites of infected mosquitoes has been demonstrated experimentally in *Macaus simiens*, using infected *Stegomia fasciata* and *Culex pipiens*.

According to Dr. Molinelli and coworkers' report on a large slaughterhouse in Buenos Aires, sacrificing some 4,800 cows, 3,000 sheep and 2,500 hogs, serum agglutination tests were positive in 6.67 and 4.48% of the hogs and cows, respectively, with no positive tests in the sheep. Of the 2,000 employees, 7 of the 18 medical veterinarians had the disease (of the infected, 1 worked with cows and the rest with hogs, one of the latter succumbing); 14 (6 of whom worked with cows

and the rest with hogs and other animals) of the 52 assistant veterinary inspectors had the disease. In the rest of the employees, 53, or 3.02%, had brucellosis. In the city of Buenos Aires, 19.44% of the raw and 2.77% of the pasteurized milk contained *Brucella abortus*, according to the Lab. of Bacteriology, Dept. of Agr.

Survival periods for the organism are discussed. Recommendations for the control of milk and milk products were made, including an official control of pasteurization, which has not been adequate, as shown by the presence of brucella in pasteurized milk. Compulsory testing of herds for Bang's disease was not mentioned.

L. S. Olsen

581. Effect of gonadal hormones on experimental infection of rats with *Brucella abortus*. L. H. PUGH. N. J. Agr. Expt. Sta., New Brunswick. Proc. Soc. Exptl. Biol. Med., 68, 3: 591-592. July-Aug., 1948.

One-half of 15 immature intact male rats and 22 immature intact female rats were injected subcutaneously with 2.5 mg. of testosterone propionate 6 hr. before infection. All of the rats were infected with 3,800 million *Brucella abortus* organisms via the intraperitoneal route. After 5 d. the mortality rate of the non-injected animals was 100%; injected males, 87.5%; and injected females, 63.6%. It was concluded that testosterone propionate significantly increased the resistance of rats to experimental *B. abortus* infection.

R. P. Reece

CHEESE

A. C. DAHLBERG, SECTION EDITOR

582. Expansion Seen for Cottage Cheese. Anonymous. Milk Plant Monthly, 38, 5: 30-31, 43. May, 1949.

In the past 10 yr. the national per capita consumption of cottage cheese has risen from 0.33 lb. to 2 lb. With this increased consumption there appeared a nomenclature for the types of cottage cheese so confusing that attempts were made to classify the varied forms. The following six categories were proposed: (a) medium sized curd lightly creamed, (b) medium and large curds heavily creamed, (c) small curd heavily creamed, (d) small curds heavily creamed with free cream showing on the bottom of the container, (e) small curds floating in added cream, and (f) very fine curds creamed to approach the texture of cream cheese. Although these varied types have added confusion to our nomenclature they have aided in increasing consumption of the product due to varied local and

regional tastes.

J. A. Meiser, Jr.

Also see abs. no. 614, 615.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

583. Dehydrated Animal Products. 2. Dried Milk. J. A. PEARCE, Natl. Research Lab., Ottawa, Can. Food in Canada, 8, 4: 14-18. Aug., 1948.

Methods of manufacturing dried milks are described and current research being conducted by the National Research Lab. is reviewed. The effect of such factors upon quality as promptness of cooling the powder, exposure to light, storage temperature, gas storage and compressing the powder into blocks is dealt with.

O. R. Irvine

584. Dehydrated Animal Products. 3. Milk Products. J. A. PEARCE, Natl. Research Lab., Ottawa, Can. Food in Canada, 8, 10: 14-17, Oct., 1948.

A brief description is given of a dried milkshake mix having the following composition: fat, 14%; protein, 31%; carbohydrate, 46%; ash, 6.9%; and moisture, 2.5%. This was made entirely from milk products. Attempts to combine egg products in the mix resulted in unsatisfactory flavors when stored. Results in terms of cake volume and foaming volume are given where the German products "Milei G" and "Milei W", made from milk, were compared to fresh eggs, sugar-egg powder and plain egg powder. In both respects the German egg substitutes were much inferior. Investigations were made on methods of drying whey and on the baking properties of whey powder. The results indicate the possibilities of using this product in baked goods, although strong odors and flavors are a problem. These were somewhat reduced by neutralizing the whey prior to condensing and drying.

O. R. Irvine

585. Process of Making Whey Food Products. R. E. MEADE AND J. M. STRINGHAM (assignors to Western Condensing Co.). U. S. Patent 2,465,905. 6 claims. March 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1524. 1949.

A firm plastic mass or gel, suitable for animal or poultry feeding, is made by fermenting pasteurized whey with propionic and lactic acid type organisms, blending with partially delactosed whey, heating to at least 160° F. and concentrating to form a final product containing from 40 to 60% solids.

R. Whitaker

586. Method of Making Novel Products from Whey. R. E. MEADE AND P. D. CLARY, JR. (assignors to Western Condensing Co.). U. S. Patent 2,465,906. 5 claims. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1524. 1949.

The browning and staling of dried whey is prevented by passing the liquid whey prior to drying through resinous decationizing and deacidifying agents to reduce the ash content and such protein decomposition products as peptides and amino acids. R. Whitaker

587. Method of Making Lacteal Food Products. R. E. MEADE AND P. D. CLARY, JR. (assignors to Western Condensing Co.). U. S. Patent 2,465,907. 5 claims. March 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1524. 1949.

A powdered infant food made from cow's milk and having the average composition of breast milk when diluted, is made by drying heat treated cream with the liquid whey powder described in Abstract 586 (Pat. 2,465,906). R. Whitaker

588. Preparation of Dried Protein Products. E. W. HOPKINS (assigned to Armour and Co.). U. S. Patent 2,465,875. 5 claims. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, 620, 5: 1517. 1949.

Acidic proteinaceous materials such as fermented milk, whey, eggs and the like are neutralized to pH 5.65 to 6.5 with a non-volatile alkali, such as NaHCO_3 , then to pH 7.0 to 8.5 with a volatile alkali, such as NH_3 , and then dried. The pH of the reconstituted product is close to the neutral point. R. Whitaker.

589. Method of sealing empty cans. P. T. LEMMEL (assignor to the Borden Co.). U. S. Patent 2,471,332. 2 claims. May 24, 1949. U. S. Pat. Office, 622, 4: 1212. 1949.

The vent hole in evaporated milk cans is plugged with a waxy material until ready for filling to keep the interior of the can clean and to prevent corrosion of the unplated vent hole edges. R. Whitaker

590. Frozen Concentrated Milk. C. D. COLVARD, Catawba Dairy, Inc., Hickory, N. C. AND W. M. ROBERTS, N. C. State College, Raleigh. Milk Dealer 38, 7: 46, 100-106. Apr., 1949.

The following conclusions are drawn concerning the processing and storage of frozen milk: (a) Milk concentrated to a ratio of 3:1 was successfully stored at -12° for 20 wk. (b) The addition of 0.20% sodium citrate, 0.075% sodium hexametaphosphate or chocolate syrup prolonged the

period of storage at 0° F. (c) Storage of frozen concentrated milk at -12° F. was more effective in preventing protein flocculation than at 0 or 10° F. (d) Samples stored at -12° F. retained their flavor for a longer period of time than those stored at 0 and 10° F. (e) Although the addition of either 0.20% sodium citrate or 0.075% sodium hexametaphosphate retarded protein flocculation, these quantities were sufficient to produce a salty flavor. (f) Concentrated milk to which chocolate syrup had been added maintained flavor and protein stability for 11 wk. or for 8 wk. longer than the control when stored at 0° F. (g) Fat de-emulsification was not a serious defect in milk concentrated to a 3:1 ratio and frozen statically. (h) A gravimetric method, which appears to be more satisfactory than the volumetric method, was developed for measuring protein stability.

C. J. Babcock

Also see abs. no. 577, 599.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

591. Isolation of *Brucella* from Apparently Healthy Individuals. L. V. McVAY, F. GUTHRIE, I. D. MICHELSON AND D. H. SPRUNT, Univ. of Tennessee, Memphis. Proc. Soc. Exptl. Biol. Med., 69, 3: 607-608. Dec., 1948.

Enlarged prostates and fibrosed fallopian tubes were cultured for brucella since they are known to multiply in macrophages and fibroblasts. Thirty-four prostates were cultured and from these cultures *Brucella abortus* were isolated in two instances and *Brucella melitensis* in one. Forty-three fallopian tubes were cultured and from one of these *B. melitensis* was isolated. In all four cases there was a history of country life, contact with cows and other farm animals, consumption of raw milk and a clinical record of illness compatible with brucellosis. Blood agglutinins for brucella were absent in all four cases; however, their skin gave strongly positive reactions with brucella antigen. R. P. Reece

592. Electron Microscope Studies of Bacteriophage Active Against *Streptococcus lactis*. C. E. PARMELEE, P. H. CARR AND F. E. NELSON. J. Bact., 57, 4: 391-397. Apr., 1949.

Here are presented 16 excellent reproductions of electron micrographs of gold-shadowed specimens. Details of preparation are given. Normal cells of *Streptococcus lactis* are shown, with and without the presence of phage particles. The particles are sperm-shaped, 220 $m\mu$ long, with a head diameter of 70 $m\mu$, and a tail 30 $m\mu$ wide and 150 $m\mu$ long. Two strains of phage

from New Zealand, four from England, one from Canada and two isolated at the Iowa Agr. Expt. Station are indistinguishable with respect to shape and size.

The bacterial cells in the presence of homologous phage tend to become elongated and to burst. Possible stages in the process of lysis are indicated. D. P. Glick

593. A Study of Boric Acid Media for the Separation of *Escherichia* and *Aerobacter*. C. F. POE AND L. W. CHARKEY, Univ. of Colorado, Boulder. J. Bact., 57, 3: 386-387. Mar., 1949.

Boric acid in culture media showed greater inhibition toward strains of *Aerobacter* than toward strains of *Escherichia*. However, because of overlapping results secured from individual strains of each genus, boric acid media are not suitable as differential media for the two genera.

D. P. Glick

594. The Fermentation of Alpha-methylglucoside. DOROTHEA E. KLEMMER AND C. F. POE. Univ. of Colorado, Boulder. J. Bact., 57, 3: 384-385. Mar., 1949.

Alpha-methylglucoside is fermented about equally by gas-producing strains of *Escherichia coli* and by strains of *Aerobacter aerogenes*. Gas-forming strains of *E. coli* produced more acetic, formic, lactic and succinic acids than did the non-gas-forming strains; e. g., the gas-forming strains produced 12 mg. of formic acid /g. of glucoside as compared with 1.6 mg. for the non-gas-formers.

D. P. Glick

595. The Action of Phenol-Bile Media on the Genera *Escherichia* and *Aerobacter*. C. F. POE AND RUBY J. O'KELLY, Univ. of Colorado, Boulder. J. Bact., 57, 3: 385-386. Mar., 1949.

Solid media containing bile and phenol did not serve to differentiate between *Escherichia* and *Aerobacter* species. However, these cultures were not inhibited by media containing as much as 11% bile and 0.1% phenol, whereas sporeforming lactose-positive aerobes were inhibited by 5% bile and 0.05% phenol. Several anaerobes grew well in media containing 15% bile and 0.2% phenol.

D. P. Glick

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

596. Oxidized Flavor in Milk and Cream. T. L. FOSTER, Univ. of Manitoba, Winnipeg. Milk Plant Monthly, 38, 5: 28-29, 37. May, 1949.

The relation of Cu contamination, ascorbic acid, dissolved oxygen, bacterial growth, homogenization and pasteurization temperature to oxidized flavor are discussed. A discussion of the value of certain antioxidants in retarding this off-flavor is also included. J. A. Meiser, Jr.

597. Method of Forming Protein Compositions. J. R. CALHOUN AND T. M. BUZZO (assignors to The Borden Co.). U. S. Patent 2,469,546. 12 claims. May 10, 1949. Official Gaz. U. S. Pat. Office, 622, 2: 463. 1949.

Casein, free from air bubbles, is prepared by applying about 25 in. of vacuum for 30 min. to a casein suspension in water, then adding caustic soda and gradually heating to 170° F. to dissolve the casein.

R. Whitaker

DAIRY ENGINEERING

A. W. FARRELL, SECTION EDITOR

598. Mechanical Can Washing. C. B. SHOOREN, Klenzade Products, Inc., Beloit, Wis. Milk Dealer 38, 7: 76-78. Apr., 1949.

A method is outlined for using acid to remove lime deposits from straightline can washers. The alternate use of acid and alkaline detergents is recommended for best can washing results. A few simple rules are given which should be observed in operating any type of straightline can washer.

C. J. Babcock

599. Spray Drier Apparatus. J. M. HALL (assignor to Drying and Concentrating Co.). U. S. Patent 2,469,553. 8 claims. May 10, 1949. Official Gaz. U. S. Pat. Office, 622, 2: 464. 1949.

A cone shaped spray drier, suitable for dehydrating milk and other products, is so shaped and arranged that heated air fed in the top spirals downward to fan blades which reverse the air flow, causing it to flow upward in the center, where it is discharged and may be collected, reheated and again circulated. The liquid product is atomized by a centrifugal wheel and the dried product removed from the bottom of the cone. The air is heated in the upper portion of the chamber.

R. Whitaker

600. Scraper for Freezing Apparatus. C. ERICKSON AND E. SPELLMAN. U. S. Patent 2,470,691. 8 claims. May 17, 1949. Official Gaz. U. S. Pat. Office, 622, 3: 900. 1949.

A scraper assembly for an ice cream freezer which provides for two easily detachable blades pivoted on a rotating member attached to a central shaft is described.

R. Whitaker

601. The Use of Ultrasonic Energy in Agriculture. L. E. CAMPBELL AND L. G. SCHOENLEBER, USDA, Beltsville, Md. *Agr. Eng.* 30, 5: 239-41. May, 1949.

Ultrasonics refers to sound radiations above the normal audible limit and is differentiated from "supersonics" which denotes velocities greater than the speed of sound in air. Ultrasonics range in frequencies from 20,000 cycles to about 10,000 megacycles/sec. Ultrasonic waves are generated by siren-type generators, magnetostriction generators and the piezoelectric generator.

Preliminary investigations have been made to determine possible practical applications to agriculture. Some of these were concerned with stimulation of seeds and tubers, killing of the codling moth, bactericidal treatments, production of emulsions and suspensions and the homogenization of milk. The latter is among nine fields suggested for immediate investigation.

H. L. Mitten, Jr.

602. Practical ammonia refrigeration. C. H. MINSTER, Greenbrier Dairy Prod. Co., Beckley, W. Va. *Ice Cream Rev.*, 32, 10: 46, 48, 52, 54. May, 1949.

The advantages and disadvantages of direct expansion, brine and sweet water refrigeration systems are discussed. It was calculated that a 7 x 7 in. compressor would be required to cool 1,000 gal. of milk from 85 to 38° F./hr. using direct expansion, whereas a 5 x 5 in. compressor could handle this same load if sweet water with an ice bank was used. A method is suggested for calculating the size of ice system necessary for use with sweet water. Hold-over ice bank systems may be purchased commercially, or the unit may be constructed. If they are to be constructed, certain points which should be observed if the system is to operate efficiently are discussed.

W. J. Caulfield

603. Basic Principles of Piping (a Review of Fundamentals). H. VETTER, Consulting Eng., Los Angeles, Calif. *Heating, Piping Air Conditioning*, 21, 5: 87-90. May, 1949.

This article concerns refrigerant piping, which can be divided into liquid line between condenser and evaporator, low pressure vapor piping between evaporator and compressor, and the high pressure vapor piping between compressor and condenser.

Efficiency of a compressor is affected by pressure drop between evaporator and compressor and between the compressor and condenser. Vapor has its highest density at saturation and

should enter the compressor near saturation. Pressure drop represents power loss due to friction and friction causes superheat. The larger the pipe line to the compressor the less the friction and the higher the compressor efficiency.

Condenser types discussed are atmospheric, double pipe, horizontal shell and tube, vertical shell and tube, and evaporative. Evaporators are discussed briefly.

Low pressure vapor piping is the most important section in the system. Liquid refrigerant carried out of the evaporator in wet vapor is a definite loss. Low pressure vapor piping should slope back to the evaporator to return as much as possible of the liquid carried over during sudden load variations. If adequate vaporizing space has not been provided in the evaporator, a separator should be installed.

High pressure vapor lines carry superheated vapor and may, therefore, be level. When they run through low temperature air, they should slope toward the condenser. All vapor piping, high or low pressure, should be arranged to avoid traps where oil or liquid refrigerant can collect.

H. L. Mitten, Jr.

604. Fuels and Firing. Part 2. P. SWAIN, I. ROWLEY, J. MCCABE AND B. SKROTZKI, McGraw Hill Pub. Co., N. Y. *Operating Engineer*, 2, 5: 19-34. May, 1949.

This article describes firing equipment used with small and medium boilers. It is well illustrated to show the operation of such devices as atmospheric gas burners, low pressure gas burners, air and steam atomizers for oil, rotary cup gas-and-oil burners, overfeed stokers for coal, and many others.

Gas comes ready to burn if properly mixed with air. Yellow flames indicate "cracking" which means that the hydrogen portion of the gas is burned first and the carbon is freed. Increase of primary air will cause an increase in gas combustion with a shorter flame free from yellow color.

Oil burners must prepare the fuel for burning since a liquid burns only as a gas. The oil burner may vaporize the fuel oil or atomize it. Vaporizing burners are limited in the range of fuel they can handle. Atomizing burners break the oil into a fine mist by using steam or air under pressure, by forcing oil under pressure through a nozzle, or by use of centrifugal force. Oil burner design must be such that oil is fired in a fine mist and that yellow flames do not impinge on water-cooled surfaces.

Coal stokers, grates, spreaders and furnaces are discussed in detail.

H. L. Mitten, Jr.

605. How to Service Package Boilers. K. STEINER, C. Hoffberger Co., Baltimore, Md. Heating, Piping Air Conditioning, 21, 5: 83-86. May, 1949.

Package boilers combine boiler, burner and mechanical draft system into a single piece of equipment. They are made for the automatic burning of gas or oil. They require a minimum of headroom and need only a smooth concrete slab for a foundation. Package boilers are rated according to their greatest possible output and have little or no reserve as do conventional boilers. Because of this they must be selected on the basis of a generously estimated load, pickup and radiation loss, and future expansion needs.

Since burners generally are connected at the factory, field installations require only connection of fuel lines, water make up lines, condensate feed lines, steam line, blow-down pipe and electrical lines. After the boiler is installed and ready to start, it should be checked and tested before being placed into service. Checks should include ignition and flames and stack temperature. Normal loads may run exit gases between 500 and 600°. Heavy loads may cause the temperature to be above 600°. Temperatures higher than normal for a given load condition indicate the flame is too long, or the tubes are sooted or the baffles between the passes are leaking. Procedure for adjustment of fire varies with the type of unit and burner. Other maintenance items to be observed are treatment of feed water, periodic internal inspection, cleaning of oil heaters and the checking of all safety controls.

H. L. Mitten, Jr.

606. Know Feedwater-Treating Costs Before You Buy. V. J. CALISE, Liquid Conditioning Corp., Linden, N. J. Power, 96, 6: 100-4. June, 1949.

Items which make up the yearly cost of operating feedwater-conditioning equipment are initial investment for equipment and erection (usually amortized over a 5 to 15 yr. period), cost of chemicals required, cost of labor for operating the treatment plant, maintenance costs, and cost of fuel for unrecovered heat in waste blowoff or cooling water. After fuel cost, cost of chemicals is highest. Chemical cost can be reduced by application of chemical and mechanical skill.

The treating process and equipment selected must produce an effluent feedwater that will eliminate the common problems caused by the presence of dissolved mineral impurities, problems such as scale formation, corrosion of boiler drum and tubes, silica deposits, corrosion of condensate return lines, carry-over of solids into stream and

embrittlement of boiler drum and tubes.

The methods for treating raw make-up water are discussed. Zeolite and hot-process softening are reviewed. Chemical equations are given for the common reactions in water treating. Tables presented compare chemicals as related to effect on hardness, impurities after treatment, analysis of effluents and cost of chemicals.

H. L. Mitten, Jr.

607. A Dynamometer for Determining Depth of Freezing in Foods. H. TESSIER, Natl. Research Lab., Ottawa, Can. Can. J. Research, 27F, 2: 47-48. Feb., 1949.

A hand operated dynamometer, designed to determine depth of freezing in frozen foods, such as meat, poultry and eggs, measures the force required to drive a pointed rod through a sample of the product. Readings are indicated on a pressure gauge and may vary from 0 to 160 lb.

O. R. Irvine

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

608. Balance Your Business As Well As Your Books. F. MERISH. Milk Plant Monthly, 38, 6: 50-53. June, 1949.

Although the balancing of books is an essential item to any plant owner, the results should not indicate a mathematical balance where debits equal credits but should indicate certain fundamental business ratios. These ratios are (a) current assets to current liabilities, (b) liabilities to net worth, (c) net worth to fixed assets, (d) net sales to net worth, (e) net profit to net worth, (f) fixed assets to current assets, (g) net profit to total assets, (h) net sales to receivables, (i) net profit, to sales and (j) cash and receivables to current liabilities. Since ratios are the best yardsticks for measuring managerial efficiency, their use will show whether one is maintaining the proper balance between the operating and financial elements in business.

J. A. Meiser, Jr.

609. Are Your Depreciation Reserves in the Safety Zone? A. C. KIECHLIN, Public Accountant. Ice Cream Rev., 32, 10: 108, 110, 112, 114, 116. May, 1949.

Due to increased construction and equipment costs, depreciation reserves are apt to be inadequate to cover replacement costs when needed. Plants are urged to take immediate steps to correct inadequate depreciation reserves by setting up a special account under the name "Reserve

for increased cost of replacement". Such an account will cushion the increased replacement or construction costs when encountered.

In purchasing new equipment, depreciation rates should be set carefully and watched to determine whether any adjustments are necessary. Increased deductions on income tax returns may be allowable if they can be justified. A complete set of records on each unit or group of similar units of equipment should be kept as an intelligent means of setting up depreciation rates and in justifying changes in the rate of depreciation if necessary.

The straight line method of computing depreciation is recommended as the simplest to compute and the one preferred by the Treasury Department for income tax purposes. In this method, the estimated salvage value of the equipment is deducted from its original cost and the difference written off at a uniform rate each year during the estimated useful life of the equipment.

Increased charges for depreciation or construction necessitated under present economic conditions should be considered as a part of the production cost. This added cost, therefore, should be reflected in the selling price of the product so that all customers will pay their share of this expense. Too many plants are now absorbing this cost without knowing it because of inadequate depreciation reserves. W. J. Babcock

610. Delivery Cost Control. A. E. FRIEDGEN, A. E. Friedgen, Inc., New York City. *Milk Dealer*, 38, 7: 42-43. Apr., 1949.

Efficient maintenance and effective cost control can be attained only by the use of detailed cost reports for each individual truck. The average "cost per mile" for fleet is a poor yardstick of route-truck efficiency. A chart is presented which shows the operating cost per mile of 28 fleets varying in size from 11 to 131 trucks. The average cost ranges from about 14 cents for a fleet of 35 trucks to 3.9/mile for a fleet of 23 trucks. The 35 trucks averaged 27 miles/d., with an individual route mileage varying from 23 to 60 miles. The 23 trucks average 84 miles/d. with an individual route mileage varying from 12 to 130 miles. These mileage variations also mean a variation in the cost/mile for the individual trucks in the fleet. Therefore, the cost/mile for the entire fleet is practically useless as a means of securing lower cost and efficiency for individual trucks. C. J. Babcock

611. Two Studies of Milk Distribution Costs and Profits in the New York Market. L. SPEN-

GER, Cornell University. *Milk Dealer* 38, 7: 50, 132-142. Apr., 1949.

An Economic Study of the Operations of Six Leading Milk Companies in the New York-New Jersey Metropolitan Area, 1941-48, directed by the author is compared with *An Analysis of the Spread Between Farm and Consumer Milk Prices in New York City Under Present Practices, 1948*. The latter analysis was sponsored by the State Temporary Comm. of Agr. and directed by Dr. C. E. Young, Dean of the Graduate School of Purdue Univ. Findings of the two studies as to unit costs for various products are not directly comparable because of the difference in time and the marked changes in prices, wages and other cost factors between 1944 and 1948. The two reports are, however, in complete agreement that significant reductions in the spread between the prices paid by consumers and the prices received by farmers can be achieved only by reducing the cost of marketing services. C. J. Babcock

612. Work Simplification in the Ice Cream Plant. R. A. BAER, Bowman Dairy Co., Chicago, Ill. *Ice Cream Rev.*, 32, 10: 122, 124, 126, 128. May, 1949.

Work simplification has for its objective the production of a better product at a lower cost. At the same time, such a program should increase the satisfaction of the workers in the jobs they are doing. It is designed to eliminate waste steps or operations which contribute nothing to the accomplishment of the job.

The five basic steps involved in work simplification are: (a) Select the job to be improved. (b) Prepare a flow process chart. This involves breaking the job down into its component parts and making an exact record of every detail of the job under study in the order in which each occurs. (c) Analyze and question each step in the operation to determine whether it contributes anything to the accomplishment of the job. Each non-essential operation should be eliminated. (d) Develop a better method. Once the non-essential operations have been eliminated, then a new sequence of operations must be developed so that each will contribute directly to the accomplishment of the job. (e) The final step is to apply the new method which has been developed. This involves the human element and necessitates securing the active cooperation and participation of those involved with the job.

The ideas of the employees throughout the organization are invaluable and should be obtained, otherwise a vast fund of ideas is being wasted. When employees are approached for suggestions and ideas and given suitable recogni-

tion for their contributions, their active participation in the program usually is assured.

Work simplification offers a means of gaining a competitive advantage by producing a better product at a lower cost. W. J. Caulfield

613. Building Business With Sales Contests. F. MERISH. *Milk Plant Monthly*, 38, 5: 34, 36-37. May, 1949.

Factors to be considered in planning and conducting a successful sales contest are purpose, scoring, quotas, awards and dramatization of the contest. These items, coupled with a well worked out plan for maintaining interest, will do much in fostering any contest. J. A. Meiser, Jr.

614. Baseball Contest Spurs Cottage Cheese Sales. T. KNIGHT. *Milk Plant Monthly*, 38, 6: 63-64. June, 1949.

Routemen are divided into 2 teams and each man assigned a ball. For each carton of cottage cheese sold, each player receives 5 points. When a total of 400 points is scored by a routeman, he receives credit for a home run and the contest is renewed. At the end of 6 wk. the winning team is given an evening of free entertainment. To obtain new customers, a bonus of \$2.00 for each new customer over 3 during a 1 mo. period is given. This plan may be used year-round but must be tied up with the current popular sport. J. A. Meiser, Jr.

615. A Cottage Cheese Drive. H. FLAGG. *Milk Plant Monthly*, 38, 5: 76. May, 1949.

For every pound of cottage cheese sold, one cent is placed in a kitty which is split at the end of the contest, 75% going to the top routeman and 25% going to the runner-up. Although the contest provides little cash gain to the participants, it does produce enthusiasm for increasing sales. J. A. Meiser, Jr.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

616. The Nutritive Value of Nitrogenous Compounds for Ruminants. I. The Nutritive Value of Urea as a Protein Supplement. C. J. WATSON, J. W. KENNEDY, W. M. DAVIDSON, C. H. ROBINSON AND G. W. MUM, Dept. of Agr., Ottawa, Can. Sci. Agr., 29, 4: 173-184. Apr., 1949.

The value of urea as a source of protein for ruminants was investigated by means of feeding and slaughter trials with 30 head of beef calves

and 60 lambs over a 40 to 50 wk. trial feeding period.

In the case of the calves, those on the low-protein basal ration made small live weight gains, showed practically no deposition of protein or ash, but did show some increase in fat. Those receiving urea made relatively good gains in live weight and body nutrients. Those receiving the casein (positive control) made appreciably better gains in live weight, body protein and ash than those receiving the urea. The gains in body fat were of similar order for both urea and casein.

The gains in total weight of carcasses, and gains in weight of protein, ash and water were greater for those receiving casein than for those receiving urea.

The section of the experiment dealing with sheep, while confirming the above results, did not allow drawing conclusions regarding urea, since the sheep failed to consume much of the ration in excess of their maintenance requirement.

O. R. Irvine

617. The Nutritive Value of Nitrogenous Compounds for Ruminants. II. The Formation of Body Nitrogen from Urea Labeled with the Isotope N^{15} . C. J. WATSON, W. M. DAVIDSON AND J. W. KENNEDY, Dept. Agr., Ottawa, Can. Sci. Agr., 29, 4: 185-188. Apr., 1949.

To determine whether urea was actually metabolized by ruminants, sheep on a low protein basal ration were given gelatine capsules containing urea. In the case of one group, the urea nitrogen was 30% N^{15} . After 4 d. feeding and the administration of 10 to 12 g. of urea the animals were killed and the blood, liver and kidney proteins separated from the non-protein nitrogen by trichloroacetic acid. Nitrogen gas recovered from the protein was analyzed for its N^{15} isotope concentration by the Washington Bur. of Standards mass spectrometer. Results indicated that those proteins contained N^{15} in excess of normal abundance and it is concluded that nitrogen urea is utilized by ruminants in the formation of body proteins. O. R. Irvine

618. The Nutritive Value of Nitrogenous Compounds for Ruminants. III. Synthesis of Urea Containing N^{15} . L. C. LEITCH AND W. M. DAVIDSON, Natl. Research Lab., Ottawa, Can. Sci. Agr., 29, 4: 189-190. Apr., 1949.

A description is given of the methods and equipment used in synthesizing urea from diphenyl carbonate and ammonium nitrate. The ammonium nitrate contained 32 atom per cent excess N^{15} when purchased. O. R. Irvine

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

619. Seeing is Believing at a Mechanical Milker Clinic. P. R. ELLSWORTH, Ohio State Univ., Columbus. Milk Plant Monthly, 38, 6: 80-82. June, 1949.

Producers bring in their own milking machines for inspection, cleaning and replacement of worn parts. The machines are placed on a "wash line" and each owner follows his milker watching the cleaning and adjusting operations as they take place. This clinic aids fieldmen in the fight for clean milk from clean utensils, provides a thorough cleaning since cleaners, brushes and plenty of hot water is available, enables dairy specialists not representing commercial organizations to contact producers, replaces faulty parts and enables producers to talk over mutual problems.

J. A. Meiser, Jr.

620. Machine Milking Plant. E. G. REDIN AND K. E. OLANDER (assignors to Aktiebolaget Manus). U. S. Patent 2,469,519. 6 claims. May 10, 1949. Official Gaz. U. S. Pat. Office, 622, 2: 456. 1949.

Several carriages, each carrying a manifold, 4 teat cups and connecting hoses, travel along a rail over the cows in a milking barn. A permanent vacuum line and a sanitary milk line adjacent to the rail, provide suction for the operation of the milking unit and a means of removing the milk to a collecting tank. Attachments are provided at each stall for connecting the milker to the suction and milk lines.

R. Whitaker

621. Mechanical Milking Apparatus. C. G. HOWSE. U. S. Patent 2,470,169. 9 claims. May 17, 1949. Official Gaz. U. S. Pat. Office, 622, 3: 767. 1949.

Two rubber hoses, one for supplying vacuum and one for conducting the milk, are attached to this milking machine teat cup. The cup is operated by the pulsations of the vacuum produced in a spring-loaded valve arrangement adjacent to the cup.

R. Whitaker

622. Refrigerated Milker Pail. T. J. PFETCHER. U. S. Patent 2,470,520. 4 claims. May 17, 1949. Official Gaz. U. S. Pat. Office, 622, 3: 855. 1949.

This milk pail, suitable for collecting the milk delivered by a milking machine or by hand, consists of a funnel shaped insert leading to a tube which conducts the warm milk to the bottom of the pail, where it spreads out in a thin layer between a flange attached to the tube and the

bottom of the pail. The tube and pail bottom are both refrigerated by a circulating cooling medium.

R. Whitaker

623. Means for Milking Domestic Farm Animals and for Temporarily Storing Milk and Cooling It. G. R. DUNCAN. U. S. Patent 2,470,979. 11 claims. May 24, 1949. Official Gaz. U. S. Pat. Office, 622, 4: 1119. 1949.

A movable container, traveling on rails between 2 rows of cows standing back to back, carries milk cans which receive the milk directly from the milking machines. The milk in the cans is cooled and stored at low temperature by means of a mechanical refrigeration unit built in the can holding container.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

624. Emulsifiers are Useful. W. E. SNYDER, Univ. of Wisconsin, Madison. Milk Plant Monthly, 38, 6: 30-33, 43-44. June, 1949.

Importance, need and action of emulsifying agents in the manufacture of high quality ice cream are discussed. Formulae illustrating common emulsifiers such as lecithin, mono-glycerides, di-glycerides, tri-glycerides, Span 60 (sorbitan mono-stearate) and Tween 60 (polyoxyalkylene derivative of Span 60) are included. Over-all effectiveness of an emulsifier depends on its ability to (a) disperse itself in the water phase of the mix, (b) be absorbed on the surface of the fat globule, (c) lower interfacial tension between the fat and water phase of the mix, (d) absorb water, thus providing hydrophilic properties to the fat globule, and (e) cling to the fat globules during the routine processes of freezing, hardening and aging of ice cream.

J. A. Meiser, Jr.

625. Confection Product. E. M. KENNEDY. U. S. Patent 2,464,515. 3 claims. Mar. 15, 1949. Official Gaz. U. S. Pat. Office, 620, 3: 884. 1949.

Two frozen confections are held on the tines of a forked stick.

R. Whitaker

Also see abs. no. 600, 612.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

626. Helpful Ideas for Your Plant. T. KNIGHT. Milk Plant Monthly, 38, 5: 80-81. May, 1949.

Simple inexpensive accessories that save a plant money are stainless steel trays for homogenizer valves, stainless steel guards for glass thermometers, automatic soap dispensers for lubricating bot-

tle conveyor lines, and float controlled reservoirs for controlling the alkalinity of can washers.

J. A. Meiser, Jr.

627. Quick Frozen Homogenized Milk. F. L. CROWLEY (assignor to Crowley's Milk Co.). U. S. Patent 2,470,020. 1 claim. May 10, 1949. Official Gaz. U. S. Pat. Office, 622, 2: 582. 1949.

Milk is prepared for storage in the frozen state by treating it as follows: clarification, homogenization, deaeration by a vacuum treatment, concentration at 120° F. under vacuum, pasteurization for 30 min. 165 to 185° F., packaging and sealing under vacuum at pasteurization temperature, cooling to 60° F. and then quick freezing at about -20° F. R. Whitaker

Also see abs. no. 596, 602, 611.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

628. Thiouracil and mammary gland growth. J. J. TRENTIN, V. HURST AND C. W. TURNER, Univ. of Missouri, Columbia. Proc. Soc. Exptl. Biol. Med., 67, 4: 461. Apr., 1948.

Twenty-one young male albino rats were castrated and divided into three groups. Group 1 served as a control and about 10 d. later the rats in groups 2 and 3 were injected daily for 2 d. with 10 γ of diethylstilbestrol in oil. Group 3 had 0.1% thiouracil added to its ration. The mammary glands of group 1 showed small- to medium-sized duct systems with little or no alveolar development. Group 2 showed an increased state of mammary development with alveolar development in most animals. The glands of group 3 showed a striking advancement of mammary development over group 1 and a marked improvement over group 2. There was good duct extension with extensive alveolar development. Fifteen intact male albino mice were placed into three groups. Group 1 served as a control, group 2 was maintained for 6 wk. on a grain ration containing 1.23 mg. of dimethyl ether of diethylstilbestrol/kg., and group 3 was maintained for 6 wk. on the same level of estrogen with 0.2% thiouracil added to the feed after the

first week. The mammary glands of group 2 showed good duct extension as compared with the controls. No difference in the response to estrogen could be detected in the estrogen- and thiouracil-treated mice. R. P. Reece

629. Secretion in cow's milk of intravenously injected radioactive phosphorus P^{32} . M. KLEBER, A. H. SMITH, AND N. P. RALSTON. Univ. of California, Davis. Proc. Soc. Exptl. Biol. Med., 69, 2: 354-356. Nov., 1948.

Two lactating Jersey cows were injected intravenously with radioactive phosphorus P^{32} , one with 15 millicuries and the other with 30 millicuries. The cows were milked just before injection; 1, 2, 6 and 12 hr. after injection; and thereafter twice daily. Maximum P^{32} concentration in milk reached a peak 3 to 8 hr. after injection; this amounted to 1.21 and 1.24% of the injected dose/l. of milk. The average daily secretion of P^{32} in milk, as percentage of injected dose, for the first 6 d. after injection was 7.4, 4.9, 2.8, 2.1, 1.6 and 1.4%, respectively. In 7 d. the two cows secreted in their milk 20 and 23% of the injected P^{32} , respectively. Casein with a radioactivity of 2 microcuries/g. was prepared from milk collected during 3 d. after injection of 40 millicuries of P^{32} per cow. R. P. Reece.

SANITATION AND CLEANING

K. G. WECKEL, SECTION EDITOR

630. New Developments in Synthetic Detergents. O. M. MORGAN, Allied Chem. & Dye Corp., Buffalo, N. Y. Milk Plant Monthly, 38, 5: 52-54. May, 1949.

A very brief review of the classification and application of synthetic detergents.

J. A. Meiser, Jr.

631. Dairy Plant Housekeeping. I. E. PARKIN, Penn. State College, State College. Milk Plant Monthly, 38, 5: 69-71. May, 1949.

A discussion of good housekeeping and responsibility for it. J. A. Meiser, Jr.

Also see abs. no. 598, 619.

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEWS

632. Elementary experiments in dairy bacteriology. R. N. Doetsch and M. L. Speck. Burgess Publishing Co., Minneapolis, Minn. 62 pp. \$1.75. 1949.

This manual presents in logical sequence a series of experiments designed for a first course in Dairy Bacteriology for students with a background in general bacteriology and in general chemistry. Twenty-three experiments are outlined and lists of materials required for each experiment are given in an appendix. A list of journals and books for possible collateral reading also is presented, but, with a few exceptions, these publications are not cited specifically in the different experiments. Although *Bergey's Manual* is mentioned once in the text and quite a few statements concerning classification are made, this book is not included in the list of references. Blanks are provided for recording the pertinent data from each experiment and introductory remarks are made for each experiment. In the opinion of the reviewer, the relation of utensil contamination and later opportunities for growth in the case of coliform bacteria are not presented with quite sufficient emphasis, the impression being left that these organisms are predominantly of direct grain and fecal origin. The spiral binding is a convenience. This manual should prove very valuable to those who wish to teach from a fixed laboratory manual, as it seems to the reviewer to be the best publication of this type available. F. E. Nelson

633. Advances in enzymology. Vol. IX. F. E. Nord, editor. Interscience Publishers, Inc., New York, N. Y. 760 pp. \$9.00. 1949.

This volume maintains the high standard set by previous volumes in this series. The twelve review papers cover a wide variety of topics in an authoritative and well-documented manner. The authors represent a cross-section of European and American leaders in the various fields surveyed. The author and subject indices to the current volume assist greatly in the finding of specific material. A cumulative index of all 9 volumes which have appeared to date is included.

The chapter on "Metabolism of Semen", by T. Mann, contains considerable material on bovine semen and 336 references are cited. "The Mechanism of Fertilization in Metazoa", by John Runnström, contains much material of a fundamental nature which would be of interest to those in breeding work. Other chapters of interest to those in one or more dairy fields include: "Some Aspects of Reversible Step Reactions", by L. Michaelis; "Kinetics of Biological Reactions with Special Reference to Enzymic Processes", by A. E. Stearn; "Photochemistry of Enzymes, Proteins and Viruses", by A. Douglas McLaren; "The Nature of Viruses", by M. A. Lauffer *et al.*; "Nitrogen Metabolism of Higher Plants", by H. E. Street; "Chemistry and Enzymology of Nucleic Acids", by F. Schlenk; "Principles of Enzymic Histo- and Cytochemistry", by D. Glick; and "Industrial Biosynthesis. Part I. Fats", by A. Hesse. F. E. Nelson

634. Principles of Veterinary Science. F. B. Hadley. 521 pp., 4th Ed., Ill. W. B. Saunders Co., Philadelphia and London. 1949.

This text is of special interest to pre-veterinary students and persons interested in pursuing careers in fields related to animal husbandry. The first part of the book includes a rather comprehensive picture of the anatomy and physiology of animals, and the second part deals with animal diseases. Physiology and general function are especially emphasized.

Quite a few changes have been made in this new edition. Several chapters have been revised, and one chapter, "The Animal World", is entirely new. According to the author, this chapter was added "to give an idea of the great range of animal life". Due to the decrease of importance of horses on farms today, a great deal of space formerly allotted to horses has been reduced. With the increasing interest and importance of dairy cattle, considerably more attention has been given to them.

This book is written in a manner for rapid and thorough comprehension. Anyone interested in

any phase of livestock production would find this edition of considerable benefit. All chapters are sub-headed and divided into topics of chief concern. At the end of each chapter are a number of questions, the answers to which thoroughly summarize the contents of the chapter.

T. M. Ludwick

635. Some effects of feeding iodinated casein for a long time to cattle, swine and white rats. SVEN DYRENDAAHL (English translation by Ebba Ericks-son). Royal Vet. College, Stockholm. Pp. 3-116. 1949. (146 ref.)

Thirty-eight calves and 82 Yorkshire pigs received 1 g./30 kg. live weight of iodinated casein or (Exp. 2-pigs) 1 g./100 kg. live weight daily. Three hundred rats received varying levels, and radioactive phosphorus isotope P^{32} 40 min. before being killed. Poor flesh, osteoporosis with stiffness of gait, accelerated heart and respiratory rate after exercise, increased skin and rectal temperatures and loose feces characterized treated cattle. Exophthalmus of the eyeball in cattle, muscular tremor, nervousness and irritation also were noted. Reduced blood sugar was accompanied by lower liver glycogen content, based on blood analyses and liver biopsy samples. Periodical serous nasal discharge and coughing affected treated animals. Also, they had increased appetites, were slightly heavier but stored less body fat than did the normal animals. Cattle have been on test 3.5 yr., while pigs were slaughtered at 100 to 120 kg. live weight. Physical reactions of all animals indicated desire to lose excess body heat.

Phosphorylation processes in the liver and heart in rats which had been fed 0.01 g. of iodinated casein daily for 30 d. were observed with aid of radioactive phosphoric isotope P^{32} . They were injected with 5 c. of P^{32} in glucose solution intraperitoneally 40 min. before being killed. Liver and heart were removed, placed in 15% trichloroacetic acid solution, then ground and washed in trichloroacetic acid, so that the filtrate contained 100 ml. concerning the liver and 50 ml. regarding the heart. Analyses, and determinations of impulses/min. by the Geiger-Müller counting chamber were made on parts of this solution. Phosphorylation rate of liver and heart was higher in iodinated casein-treated animals than in controls. Nipples of treated rats were enlarged.

R. B. Becker

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

636. In vitro effect of certain antibacterial agents on organisms encountered in bovine mastitis. M. E. KRAFT AND G. R. SPENCER. Univ. of

Wisconsin, Madison. Proc. Soc. Exptl. Biol. Med., 70, 1: 176-179. Jan., 1949.

Various antibacterial agents were diluted to the approximate level which could be attained for a period of 10 to 12 hr. after intramammary infusion in cows. Subtilin was effective in high dilution against gram positive organisms. Streptomycin was more effective against gram positive cultures than against gram negative organisms. *S. aureus* and *S. typhimurium* were resistant to the sulfonamides. *Str. agalactiae* and *C. pyogenes* were susceptible to sulfapyridine, sulfone, sulfadiazine and sulfamerazine. *E. coli* was susceptible to sulfapyridine, sulfadiazine, sulfamethazine and sulfamerazine, among which the last two showed great activity. Sulfamethazine, sulfadiazine and sulfamerazine were effective against *Br. abortus*. *Ps. aeruginosa* was inhibited by sulfamerazine and sulfapyridine and sulfadiazine was the most effective antibacterial agent. R. P. Reece

637. Penicillin in *Str. agalactiae* infection: Trials made in Great Britain in 1945 and 1946. Anonymous. Vet. Record, 61, 19: 235-237. May 7, 1949.

These data on field trials on the use of penicillin for *S. agalactiae* were accumulated by members of the Mastitis Conference of the Agricultural Research Council of Great Britain working at 6 different laboratories.

Penicillin Field trials were made on 1363 *S. agalactiae* infected quarters over a 2-yr. period. The examination consisted of plating 0.1 ml. of milk on blood agar plates and reading after 48 hr. at 37° C. for identification of *S. agalactiae*. A quarter was considered infected if it showed 3 positive tests, 2 of which were 1 wk. previous to treatment. Cure was considered to be effective if 7 negative tests were obtained, 3 of which were in the week after treatment and the additional 4 at weekly intervals.

Following pilot trials, it was decided to compare the efficacy of the following dosage levels and intervals in the 1st trial: 2 doses of 20,000 units with a 24-hr. interval, 4 doses of 20,000 units with a 24-hr. interval, 4 doses of 10,000 units after successive milkings, and a single dose of 100,000 units. All penicillin injections were made in 50 ml. of water and all quarters were treated. In the 2nd trial, dosage levels and intervals were modified as follows: 2 dosages of 20,000 units with a 24-hr. interval, 2 doses of 40,000 units with a 24-hr. interval, and 2 doses of 100,000 units with a 24-hr. interval. In addition, the penicillin was injected in 10 ml. of distilled water instead of 50 ml. by one of the laboratories.

Because of the larger numbers, results were expressed in terms of quarters cured, which makes

them about 10% higher than on a cows cured basis. The total results showed that one dose of 100,000 units is inadequate and gave only 58% cure; 2 doses of 20,000 units at 24-hr. intervals resulted in 80% cure and increasing the dosage level at this interval to 40,000 or 100,000 showed no advantage; 4 doses of 10,000 units at successive milkings or 4 doses of 20,000 units in a 24-hr. period were no better than 2 doses at the 24-hr. interval, all being approximately 80% effective.

R. P. Niedermeier

638. A comparison of the immunizing value in cattle of dead antigens and S. 19 Br. abortus vaccine. A. McDIARMID, Agr. Research Council, Field Station, Compton, Berks. Vet. Record, 61, 22: 305-308. May 28, 1949.

Sixty Ayrshire heifers ranging in age from 22 to 30 mo., none of which had previously been bred, were used in this study to compare the immunizing value of dead vaccines with that of the avirulent vaccine made from strain 19. These heifers were divided into 5 groups of 12 each, with group 1 serving as a control, group 2 was vaccinated with S. 19 vaccine, group 3 was vaccinated with an antigenic fraction of *B. abortus* strain 544 and the remaining 2 groups were vaccinated with dead *B. abortus* (strain 544) suspended in lanolin and liquid paraffin. In all groups except no. 4, the antigens used were prepared from an estimated 60 billion bacillary bodies, and in group 4 the dead vaccine contained 10 times this number. When the majority of the heifers were 5 months pregnant, an infective dose (130 million organisms) of virulent *B. abortus* (strain 544) organisms was given via the conjunctival sac.

At parturition, cultural and biological examinations were made of blood, cotyledon, colostrum and fetal stomach contents. Blood samples were also taken at regular intervals during the experimental period and the agglutination test run. Results of these tests are given in detail. In general, these data demonstrated that the living avirulent S. 19 vaccine is better than dead antigens in all cases, including group 4 where the organisms used to prepare the vaccine were increased 10 times. The authors suggest that one might expect a better degree of immunity by large, repeated doses of one of the dead antigens. The lanolin vaccine seemed to be the most effective of the dead antigens. R. P. Niedermeier

639. The stability of the avirulent characters of *Brucella abortus* strain 19 and strain 45/20 in lactating and pregnant cows. A. W. TAYLOR AND A. McDIARMID. Vet. Record, 61, 23: 317-318. June 4, 1949

This study consists of 2 parts. In part I, 28

non-pregnant Ayrshire and Holstein-Friesian cows that had calved once and were negative to the agglutination test were used. All but 3 of these cows were lactating at the time they were vaccinated. The subcutaneous method of vaccination was used and 8 cows received 5.0 ml. of S. 19 vaccine; 8 were given 2 inoculations at 3-wk. intervals of 45/20 vaccine, and 12 cows served as non-vaccinated controls. These cows were housed and grazed together. The cows were bred beginning 2 mo. after vaccination. Milk samples were taken at 2-wk. intervals and examined culturally and in addition biologically for the vaccinated cows. Agglutination test was run monthly. At parturition, blood samples for the agglutination test, colostrum and cervical swabs were taken for cultural and biological examination. The prepartum sampling procedure was continued for approximately 2 mo. postpartum. Results showed that no *Br. abortus* was excreted in the milk of the vaccinated cattle, and no infection appeared in the control animals from either strain 19 or 45/20.

In part II, each strain was introduced intravenously in a pregnant negative cow at sufficient dosage level to cause abortion. At abortion it was recovered by culture from the cotyledon and in as short a time as possible another pregnant negative animal was inoculated. Each strain was passaged 7 times in this manner through pregnant cattle. In this process S. 19 remained unchanged in regard to its ability to grow in air and its accepted virulence for guinea pigs. Strain 45/20 became highly virulent by the 7th passage and became CO₂ sensitive. Its inability to produce agglutinin was lost between the second and third passage. R. P. Niedermeier

640. The mucus agglutination test for the diagnosis of bovine trichomoniasis. A. E. PIERCE. Vet. Record, 61, 25: 347-348. June 18, 1949.

Results are given for mucus agglutination tests on 465 mucus samples from clean, suspect and infected herds in the British Isles. Only the 1:10 dilution reading was used. The physical properties of the mucus were observed and the sample was then classified as oestral, post oestral, vaginal, pregnant, purulent or aqueous, prior to running the agglutination test. The successful application of the mucus agglutination test, as well as the ability to detect microscopically *T. foetus* in the various types of mucus samples is discussed. The author concluded that the mucus agglutination test is more accurate than the blood agglutination test. Inasmuch as several known infected animals failed to react to the mucus agglutination test and agglutinin may persist after recovery, the direct microscopic examination still is necessary,

but based upon this data the suggestion is made that microscopic examination be applied to only those types of mucus most likely to contain the organism.

R. P. Niedermeier

641. Some observations on milk fever. A. ROBERTSON. Vet. Record, 61, 24: 333-339. June 11, 1949.

Data given include observations on 19 cases of milk fever. Blood analyses for serum Ca, Mg and inorganic P before treatment, soon after treatment and after recovery periods are given. In several of the cases when the blood Ca was brought back to the normal level by Ca borogluconate treatment, a cure was not effected and the author points out that this supports the contention that blood Ca level is not alone responsible for milk fever. Upon statistical analysis of the data, no correlation was found between blood Mg levels and the symptomatology classification in regard to excitement, coma, narcosis or alertness. An interesting review of the historical background of research on milk fever is included, as well as a lengthy discussion by research men who have worked on this disease.

R. P. Niedermeier

Also see abs. no. 634, 655, 657.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

642. Stabilization of butter. W. S. MUELLER (assignor to the U. S. of America as represented by Secretary of War). U. S. Patent 2,472,119. 16 claims. June 7, 1949. Official Gaz. U. S. Pat. Office, 623, 1: 128. 1949.

Butter is protected against rancidity and decolorization by tetrachloroparabenzoquinone.

R. Whitaker

Also see abs. no. 726.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

643. Consumer preference as related to acidity, curd size and creaming of cottage cheese. W. H. E. REID, Univ. of Missouri, Columbia. Milk Dealer, 38, 9: 43-44, 122-126. June, 1949.

Many manufacturers of cottage cheese use the acid test of the whey or of the curd as an index to when the curd has sufficiently firmed or is ready for cutting. A survey indicated that the acidity of the whey should be between 0.45 and 0.50%. Acidity of the curd varied from 0.57 to 0.65%.

The curd size varies in different regions and in some markets it is necessary to furnish both the "Popcorn Kernel" and the "Smearcase" types to satisfy cottage cheese consumers. When creaming the cottage cheese, care should be applied

to avoid damaging the curd particles. The 3 defects commonly found in body and texture are mealiness, lumpiness and a soft, pasty texture.

The physical properties of cottage cheese manufactured from skimmilk reinforced with about 50% of nonfat dry milk solids are very comparable with cottage cheese manufactured from normal skimmilk. This was evidenced by the fact that several thousand lb. of this cottage cheese received favorable reception from the consuming public.

Nonfat dry milk solids are satisfactory for making cottage cheese. A concentration of 20%, i.e., 20 lb. of nonfat dry milk solids to 80 lb. of water is recommended.

C. J. Babcock

644. It's all cottage cheese. CAROLINE B. MENUEZ, Paper Cup and Container Institute, Inc. Am. Milk Rev., 11, 5: 10, 12. May, 1949.

The results of a survey revealed wide differences in types of cottage cheese offered for sale. Cottage cheese was classified according to six different types, based upon size of curd and relative amount of creaming. Variation in preference in different localities was pointed out. Potential marketing possibilities are emphasized by the fact that the per capita annual consumption in California is 6 lb. while the national average is 2 lb.

D. J. Hankinson

645. A comparison of starters for use in Roquefort-type cheese. J. CLARKE AND N. S. GOLDING. State College of Washington. Natl. Butter Cheese J., 40, 7: 27-29, 54. July, 1949.

Carbon dioxide is a limiting factor in mold growth. Therefore, a lactic starter producing little or no carbon dioxide would appear to be desirable for use in making Roquefort-type cheese. The purpose of this study was to determine whether pure lactic cultures or those containing associate types of organisms were most desirable for this use. A comparison was made of the following starters: a commercial starter containing associate types of organisms (*Leuconostoc citrovorum* and *L. dextranicum*) and organisms of the *Streptococcus lactis* group; a pure culture of *S. lactis*; a pure culture of *S. cremoris*. Three lots of cheese were made with each of these starter cultures. Two different strains of *Penicillium roqueforti* were used.

Cheeses made with commercial starter had more abundant mold growth than that made with either of the pure cultures. Cheeses made with the pure cultures had dry textures; those made with the commercial culture had wet or leaky texture. Characteristic Roquefort flavor could not be correlated with high total volatile acidity. Cheeses made with pure cultures were criticized

for bitter taste and foreign mold flavors. The use of commercial type starter made equal or better cheese than either of the pure cultures.

H. E. Calbert

646. Report on sampling, fat and moisture in cheese. W. HORWITZ AND LILA KNUDSEN, Food and Drug Admin., Federal Security Agency, Minneapolis 1, Minn. and Washington 25, D. C. J. Assoc. Offic. Agr. Chemists, 32, 2: 303-309. 1949.

A more extensive study of the official and modified methods for fat and moisture in cheese was conducted and analyzed statistically. The experiments were designed to determine (a) the variation between laboratories, (b) the variation between collaborators within a laboratory, and (c) the variation between duplicate samples run by the same collaborator. Results obtained in over 700 determinations are plotted graphically. Analyses of variance were performed on the data for each method. In most cases the variation between laboratories contributed a significant amount of variation. The difference between collaborators within a laboratory was very significant also. Variations are shown in terms of the standard deviation and in terms of variation to be exceeded 1 time in 20 ($P=0.05$ limits).

When the A.O.A.C. method was used for determining moisture, the results obtained between laboratories had a standard deviation of ± 0.29 and $P=0.05$ limit of 0.57; the draft oven method values of ± 0.31 and ± 0.61 , respectively. Results between collaborators within a laboratory using the A.O.A.C. method had a standard deviation of ± 0.25 and a $P=0.05$ limit of ± 0.49 ; the draft oven method gave values of ± 0.27 and ± 0.53 , respectively. Results by one collaborator using the A.O.A.C. method had a standard deviation of ± 0.16 and a $P=0.05$ limit of ± 0.31 ; the draft oven method gave results of ± 0.20 and ± 0.39 , respectively.

When fat determinations were made by the A.O.A.C. method, the results obtained between laboratories had a standard deviation of ± 0.24 and a $P=0.05$ limit of ± 0.47 ; a method employing direct weighing into a Mojonnier tube gave values of ± 0.32 and ± 0.63 , respectively. Results obtained between collaborators within one laboratory, using the A.O.A.C. method, had a standard deviation of ± 0.19 and a $P=0.05$ limit of ± 0.37 ; the method employing direct weighing into a Mojonnier tube gave values of ± 0.28 and ± 0.55 , respectively. Results obtained by one collaborator, using the A.O.A.C. method, had a standard deviation of ± 0.15 and a $P=0.05$ limit of ± 0.29 ; the method employing direct weighing into a Mojonnier tube gave values of ± 0.19 and ± 0.37 , respectively.

F. J. Babel

647. Method of processing cheese and package therefor. F. M. FISHER AND H. C. HOPP (assignors to Standard Cap and Seal Corp.). U. S. Patent 2,471,867. 1 claim. May 31, 1949. Official Gaz. U. S. Pat. Office, 622, 5: 1496. 1949.

Instead of placing freshly milled curd in conventional hoops, it is placed in molds which are lined with a non-hygroscopic envelope, such as cellophane. The product in the lined mold is placed under vacuum, and the envelope sealed. Release of the vacuum causes the envelope to shrink around the curd causing it to knit together and acting as a protective coating during curing.

R. Whitaker

Also see abs. no. 655, 671.

CONDENSED AND DRIED MILK; BY PRODUCTS

F. J. DOAN, SECTION EDITOR

648. Stabilizing evaporated milk. H. E. OTTING, L. H. CHRYSLER AND E. F. ALMY (assignors to M and R Dietetic Laboratories, Inc.). U. S. Patent 2,473,493. 11 claims. June 14, 1949. Official Gaz. U. S. Pat. Office 623, 2: 607. 1949.

Evaporated milk having a total solids content greater than the usual 26% is easily sterilized without coagulation by incorporating a small portion of mineral modified milk having a Ca/P ratio of 0.15/0.75.

R. Whitaker

649. Filtrations-probleme bei der Molkeaufbereitung. (Filtration problems in preparing whey.) English summary. F. A. FRIEDEL. Die Milchwissenschaft, 3, 10: 292-296. Oct., 1948.

In order to obtain clear whey for human consumption the whey is heated to 100° C. and held for 10 to 15 min. The heat-coagulated proteins are permitted to settle, preferably at pH 4.5, during a period of 12 hr. The supernatant is filtered through a Berkefeld wash filter consisting of a layer of infusorial earth, which treatment renders the liquid clear. An illustration of a schematic procedure is given.

I. Peters

650. Korrektur der spindelanzeige bei molke. (The correction of lactodensimeter readings in whey.) English summary. G. ROEDER. Die Milchwissenschaft, 3, 11: 335-340. Nov., 1948.

The expansion coefficient was found to be the same for rennet- and acid-type whey at temperatures of 10 to 30° C. Based on the expansion coefficient of whey, the specific gravity for each temperature was calculated, taking into account the glass correction factor of the lactodensimeter.

Thus a table was formulated for the determination of the specific gravity of whey with readings taken at 10 to 30° C. corrected to 15° C. The calculated and actual values obtained were in sufficient agreement for the degree of accuracy required in this test. I. Peters

Also see abs. no. 659, 661, 668, 669.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

651. A modified frost little plate method for routine bacteriological control of milk. E. L. E. HUMPHRIES, S. Hues & Sons, Ltd., Liverpool. Dairy Ind., 14, 4: 389-391. Apr., 1949.

A description of a modification of the original Frost technique is given in some detail. The technique consists essentially of preparing a 1 to 10 dilution of the milk to be tested in quarter-strength Ringer solution in the ordinary way. A sterile platinum loop calibrated to deliver 0.01 ml. is used to measure 0.01 ml. of this dilution, and to mix thoroughly with 3 to 4 drops of molten agar previously deposited on a sterile 3×1 in. micro slide, and finally to spread the inoculated agar over an area of 4 cm.² After hardening, the slide is at once placed in a moist chamber and incubated overnight at 37° C. Next morning the slides are placed in a water-oven at 100° C., dried, washed with acetone, then water, stained with dilute aqueous methylene blue, again washed with water and returned to the oven to dry.

A description of a microscopic counting aid is given which aids in reducing fatigue as well as errors. G. H. Watrous, Jr.

652. Doppelkombinierte methode zur indirekten keimzahlbestimmung der milch. (The combination of two methods for the indirect count of bacteria in milk.) English summary. A. TODOROFF AND K. ASSENOWA. Die Milchwissenschaft, 3, 10: 300-302. Oct., 1948.

A growth medium consisting of equal parts of peptone whey agar and nutrient broth agar when used in petri plates and in deep cultures permits the development of a large variety of bacteria differing nutritionally as well as in their demand for oxygen. Incubation at 32 to 35° C. for 48 hr. is recommended. For yeasts and molds a medium consisting of equal parts of peptone whey agar and wort agar is recommended for plating, using an incubation temperature of 30° C.

I. Peters

653. Method of deflocculating bacteria. G. GREEN (assignor to Syntrol Co.). U. S. Patent 2,472,419. 5 claims. June 7, 1949. Official Gaz. U. S. Pat. Office, 623, 1: 202. 1949.

To provide a means of breaking up clumps of bacteria, prior to counting, the medium is subjected to mechanical vibration of frequencies of 3,600 to 7,200 vibrations/min. R. Whitaker

654. A note on morphological differences between strains of *Streptococcus cremoris*. H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Research Inst., Palmerston North, New Zealand. J. Gen. Microbiol., 3: 43-45. 1949.

Cultures of *Streptococcus cremoris* were grown in milk for 5 hr. at 37° C., rather than at the optimum of 30° C., and then examined under the microscope. The appearance of involution forms served as a characteristic which was considered to be significant in the identification of various strains of this organism.

J. J. Jezeski

655. Penicillin in relation to acid production in milk by starter cultures used in cheddar cheese making. H. KATZNELSON AND E. G. HOOD, Dept. of Agr., Ottawa, Can. Science, 109, 2837: 490. May 13, 1949.

The carry over of penicillin used as a treatment for mastitis into milk used for cheese may be great enough to inhibit the activity of acid producing bacteria. Pasteurization of milk failed to inactivate the antibiotic. M. Loewenstein

656. Zur taxonomie der mikroorganismen. (On the taxonomy of microorganisms.) English summary. A MEYN. Die Milchwissenschaft, 3, 10: 297-300. Oct., 1948.

A discussion dealing with the importance and method of systematic classification of microorganisms is presented. I. Peters

657. The identification of *Brucella abortus* strain 19 by dye bacteriostasis. H. B. LEVINE AND J. B. WILSON, Univ. of Wisconsin, Madison. J. Infectious Diseases, 84, 1: 10-14. Jan.-Feb., 1949.

The work was undertaken to find an *in vitro* test to replace the quinea pig virulence test for the identification of strains of *Brucella abortus* which do not require added CO₂ on primary isolation. The tolerance to 6 basic thiazin dyes was studied with 97 cultures of *Br. abortus*. The cultures included 31 cultures of strain 19 from various sources, as well as virulent, relatively avirulent and aberrant cultures of human and animal origin. The dyes used were thionin, thionine blue, methylene blue, azure A, azure B and azure C. The organisms were streaked onto plates of Bacto-Tryptose agar to which had been added after sterilization the desired amount of sterile dye solution. The criterion of differentiation was the fail-

ure of strain 19 to grow at a dye level that did not inhibit other strains.

All of the strain 19 cultures were completely inhibited by 0.4 mg. thionine blue/l. of medium. All of the virulent strains tolerated at least 50% more of the dye. For routine testing the authors recommended a dye level of 0.4 to 0.5 mg./l. and an incubation period of 5 d. J. F. Cone

658. Today's milk is safe milk. A National Dairy Council Digest. Am. Milk Rev., 11, 5: 44-45. May, 1949.

Largely because of pasteurization, disease outbreaks due to milk have declined in recent years. At the same time outbreaks due to other foods increased. Milk borne outbreaks are largely confined to small cities and rural areas where pasteurization is less common. Eradication plans for animal diseases communicable to man, such as tuberculosis and brucellosis, are important health measures. The effect of pasteurization on the food value of milk is briefly discussed.

D. J. Hankinson

Also see abs. no. 636, 637, 638, 639, 640, 645.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

659. Vergleich verschiedener fettbestimmung's methoden bei der akwendung auf milchpulver under kindernährmittel! (A comparison of various methods for the determination of fat in milk powder and baby foods.) English summary. W. MOHR AND J. HÄSING. Die Milchwissenschaft, 3, 11: 321-327. Nov., 1948.

Dried powdered whole milk, skimmilk, butter-milk, whey and cream, as well as baby foods and butter, were analyzed for fat content by five different methods. The Schmidt-Bondzynski method gave variable results, depending on the heat treatment of the respective milk powder. The Roesse-Gottlieb method showed low values with rancid fat powder but not with normal fat powder. Schlowmer carbon tetrachloride method gave variable low values, whereas the Grossfeldt pure extraction method gave accurate values, provided the extraction time was of sufficient duration. This method measures also the phosphatides and therefore gave higher values than did the Weibull-Stoldt method. The latter gave accurate reproducible values in all instances and is recommended for the determination of fat in dried milk and whey. The method is as follows: A 20 g. sample is suspended in 100 ml. cold water and 60 ml. fuming HCl (spec. gr. 1.19). This mixture, with pumice added, is heated on a water bath with intermittent stirring until foaming ceases. The container then is covered with a watch glass

and heating continued on an asbestos gauze over a direct small flame and boiled for 20 min. with intermittent stirring. Boiling water is added and the hot mixture filtered through a moistened folded filter paper of 27 cm. diam. After 3 washings with hot water and careful draining, the filter is folded, placed on a watch glass and dried for 3 to 4 hr. in a drying oven at 105° C.

The filter without thimble is placed directly into an extraction apparatus containing a pad of fat-free cotton at the bottom. Extraction in the Soxhlet apparatus is continued for 3 hr. using 175 ml. ether. The ether is evaporated and the fat is dried to constant weight at 105° C. The limits of error by this method for whole milk powder is ± 0.1 per cent. I. Peters

660. Report on the detection of added water by the serum tests. H. J. HOFFMAN, Dept. of Agr., Dairy and Food, St. Paul, Minn. J. Assoc. Offic. Agr. Chemists, 32, 2: 309-317. 1949.

Results obtained in a collaborative study indicate that the official serum methods only serve to indicate to the analyst that added water may be present in a milk sample. Milk samples falling below the present standards (38.3 sour serum, 36 copper serum and 39 acetic serum) contain added water. The methods gave no indication of the amount of added water. A suggestion was made not to prosecute for added water unless cryoscopic results were available. Data obtained by the serum methods were not uniform. It was recommended that the serum methods be confined to their present limitations, i.e., indicating the presence of suspected samples. F. J. Babel

661. Methanol extraction of lactose and soluble proteins from skim milk powder. A. LEVITON, Bur. Dairy Ind., USDA, Washington, D. C. Indus. Eng. Chem., 41, 7: 1351-1357. July, 1949.

Earlier work on the separation of soluble proteins and lactose from whey powder has been extended to fluid and dried skim milk. The proteins of spray-dried skim milk were almost completely precipitated by 62% methanol at -15° C. The dried milk-methanol mixture was centrifuged within a few minutes after mixing to remove the protein precipitate. Lactose of good quality crystallized from the filtrate during 15 hr. and was recovered by centrifuging. The protein product was soluble in water and showed no significant change in particle size distribution from that in the original milk. The protein product recovered comprised 42.2% of the solids of skim milk and it contained 74.1% protein of which 81% was casein. The influence of solvent-powder ratio, the effect of methanol concentration, of higher extraction temperatures and the properties

of the lactose and the protein complexes were determined. Best results were obtained when 20 g. of powder were extracted with 100 ml. of 62% methanol. The results indicate that, for many industrial purposes, extraction at room temperature would produce a satisfactory soluble protein product. For best results the skim milk used should be a soluble spray-dried product in which the lactose has not crystallized. The process can be applied to fluid and concentrated skim milk, but with these products there is an alcohol rectification and a serious filtration problem. A study of the extraction of the fluid skim milks yielded interesting data on the constitution of the proteins in milk.

B. H. Webb

662. Elektronen-mikroskopische Größenbestimmung der Calciumcaseinatteilchen in Kuhmilch. (Electron microscope determination of size of calcium caseinate particles in cow milk.) NITSCHMANN, Hs., Univ. of Bern, Switzerland. *Helvetica Chimica Acta*, 32: 1258-1264. 1949.

Preparations were made from skim milk diluted with 0.01 M CaCl₂ or treated with formalin and then diluted with distilled water. Simple dilution with distilled water allowed dispersion of some particle aggregates. The preparations were made on glass, gold shadowed and removed on a formvar lacquer film. The most common particle size is 80-120 m μ , with considerable numbers of particles in the 40-80 m μ and 120-160 m μ classes. Some particles are as large as 280 m μ . These values agree reasonably well with certain other values which have been reported.

F. E. Nelson

663. The binding of organic ions by proteins. Comparison of native and modified proteins. I. M. KLOTZ AND J. M. URQUHART. Northwestern Univ., Evanston, Ill. *J. Am. Chem. Soc.*, 71, 5: 1597-1603. May, 1949.

A comparative quantity study was made of the binding of a common anion, methyl orange, by a group of proteins, mostly of crystalline nature, under very identical environmental conditions. The extent of binding of methyl orange was measured by a differential dialysis technic. Bovine plasma proteins, fractions II (γ -globulin) and III-1 (β 2-globulin), did not bind methyl orange. The extent of binding by fraction IV-1 (α 2-globulin) is quite small, while the crystallized albumin fraction showed significant binding properties. Among the non-plasma proteins examined only β -lactoglobulin showed appreciable uptake of methyl orange. Modification of proteins, such as acetylation, decreases the affinity of albumin for anions. Where the number of cationic loci are not decreased, such as the conversion of the

ϵ -ammonium groups of lysine to guanidinium groups, the binding ability of serum albumin remains unaltered.

H. J. Peppler

664. Compounds with "folic acid" activity. A. Z. HOBSON, Pet Milk Co., Greenville, Ill. *Arch. Biochem.*, 21, 2: 330-334. Apr., 1949.

The activity of possible interfering compounds in the folic acid assay of milk were compared with that of pteroylglutamic acid for *Lactobacillus casei* and *Streptococcus faecalis*. Under the conditions tested, ribonucleic acid, uric acid, glutamine and orotic acid did not interfere in assays for folic acid while desoxyribonucleic acid and 5-methylthiouracil are active for *L. casei* and *S. faecalis*. The results do not explain the discrepancies observed in the folic acid assay of milk nor do they necessarily invalidate the values already reported for milk and other foods.

H. J. Peppler

665. The digestion of acetyl proteins by pancreatin. B. M. HENDRIX AND W. J. WINGO, Univ. of Texas. *Arch. Biochem.*, 21, 2: 431-36. Apr., 1949.

The digestive action of pancreatic extract upon acetylated casein, edestan and egg albumin, and their alkali-treated derivatives, was determined, and the nature of the binding of the acetyl group to the protein was studied. As much as 69% of the acetyl casein was digested, based on the amino nitrogen set free by the native protein. The digestibility of acetyl casein differed only slightly from that of its alkali-treated derivative. Less acetyl was liberated from acetyl casein by pancreatic digestion than was removed by the solution of the protein in dilute NaOH. The acetyl removed from alkali-treated acetyl casein by pancreatin amounts to less than 5.5% of the acetyl bound to the casein prior to enzyme digestion or alkali treatment. The liberation of acetic acid from acetyl proteins is believed due to esterases of the pancreatic extract. These esterases split the acetyl group bound by oxygen linkage to various groups in the protein molecule, such as the phenolic hydroxyl of tyrosine, other hydroxy-amino acids and carbohydrate groups.

H. J. Peppler

666. Process of preparing modified protein. I. A. PARFENTJER (assignor to American Cyanamid Co.). U. S. Patent 2,473,255. 3 claims. June 14, 1949. Official Gaz. U. S. Pat. Office, 623, 2: 547. 1949.

A protein of improved nutritional value is made by digesting casein with pepsin at pH range 2 to 8, until most of it is water-soluble at pH 4.6. The desired fraction is salted out with 25 to 35% by

weight of $(\text{NH}_4)_2\text{SO}_4$, then the salt is removed by dialysis.

R. Whitaker

667. A capillary-ascent test tube method for separating amino acids by filter paper chromatography. L. B. ROCKLAND AND M. S. DUNN, Univ. of Calif., Los Angeles. *Science*, 109, 2839: 539. May 27, 1949.

A rapid, convenient, capillary-ascent test tube method for separation of less than γ quantities of amino acids by filter paper chromatography is described.

M. Loewenstein

Also see abs. no. 642, 646, 648.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

668. Apparatus for the desiccation of organic substances. F. E. HURD. U. S. Patent 2,471,035. 4 claims. May 24, 1949. Official Gaz. U. S. Pat. Office, 622, 4: 1133. 1949.

Milk, fruit juices and the like are first super-cooled in a pressure tank and then sprayed into a vacuum chamber at 29 in. Hg or lower, where the product freezes in the form of extremely small droplets. The moisture sublimates as the particles fall to the bottom of the cone shaped chamber and the dry powder is removed and packaged. Heat may be applied to the nozzle to prevent freezing of the liquid on the nozzle. The chamber also is jacketed and insulated to permit control of the temperature within the chamber.

R. Whitaker

669. Spray device. R. E. MFADE, N. E. TAYLOR AND J. F. CREWS (assignors to Western Condensing Co.). U. S. Patent 2,473,035. 6 claims. June 14, 1949. Official Gaz. U. S. Pat. Office, 623, 2: 490. 1949.

The chief novel feature of this centrifugal spray wheel for atomizing fluids like milk, whey, etc. is a series of teeth on the rotating wheel upon which the material impinges and is thrown outwardly from the device in a fine mist.

R. Whitaker

670. Heat exchange system. R. E. OLSON (assignor to Taylor Instrument Co.). U. S. Patent 2,472,984. 2 claims. June 14, 1949. Official Gaz. U. S. Pat. Office, 623, 2: 477. 1949.

Details are given of a flow diversion valve and an automatic system of operation for the valve for ensuring the proper temperature of milk entering the holding tube of a high temperature-short time pasteurizer for milk.

R. Whitaker

671. Protecting cheesemakers' profits with instruments. J. MEYER, Minneapolis-Honeywell

Regulator Co., Brown Instrument Div., Philadelphia, Pa. *Natl. Butter Cheese J.*, 40, 6: 32-33, 50-51. June, 1949.

A "continuous balance" electronic potentiometer is a new device that can be used as a control instrument to prevent small fluctuations of temperature when high temperature-short time pasteurization of milk for cheesemaking is used. For smaller operations using the holding method of pasteurization, a standard dairy thermometer with built in timer, controller and signal lights now is available, serving as a means of automatic control in vat pasteurization.

H. E. Calbert

672. A guide to checking high temperature short time pasteurizer. P. J. DOLAN, JR., Calif. Dept. Agr. *Am. Milk Rev.*, 11, 5: 40-41. May, 1949.

Cardinal points in checking performance and compliance of high temperature short time units with ordinance requirements are divided into 4 phases. The 1st phase, construction and arrangement of equipment, includes suitable clearance around equipment, sanitary construction, condition of plate gaskets, raw milk surge tank location, raw milk pressure relationship to pasteurized side, characteristics of the timing pump, slope of the holding tube, recording and indicating thermometer construction, and flow diversion valve construction. The 2nd phase is thermometer and controller accuracy. The 3rd phase is thermometer temperature controller and flow diversion valve response. The 4th phase, holding time, is determined by salt solution injection into water flow, using an electrode and meter as an indicating device. Filling time for a 10 gal. can may be used as a quick check if established when the unit is operating properly. Timing should be carried out as in operation—in diverted flow and with and without homogenizer in operation. The timing pump should be sealed at its maximum speed.

D. J. Hankinson

673. Receptacle handling apparatus. I. H. KENDALL (assignor to Cherry Burrell Corp.). U. S. Patent 2,473,955. 7 claims. June 21, 1949. Official Gaz. U. S. Pat. Office, 623, 3: 870. 1949.

This device takes inverted milk cans from a can washer, turns them right end up and delivers them on a conveyor with the lids in place.

R. Whitaker

674. Ice cream machine with perforated screw agitator. G. H. G. ESPINASSE AND J. P. C. ESPINASSE. U. S. Patent 2,474,730. 3 claims. June 28, 1949. Official Gaz. U. S. Pat. Office, 623, 4: 1176. 1949.

The chief feature of this ice cream freezer is a screw conveyor type of agitator which operates within a refrigerated cylinder. R. Whitaker

675. Refrigerating-plant efficiency. J. F. HYAM, Sarnia, Ont. Power, 93, 6-A: 142. Mid-June, 1949.

An equation and an alignment chart are presented for determining the efficiency of refrigerating plants under various temperature conditions.

H. L. Mitten, Jr.

676. Good purge means more tonnage. T. G. HICKS. Operating Engineer, 2, 6: 36-7. June, 1949.

High condenser pressure, poor condenser heat transfer, increased power input and formation of explosive mixtures may indicate an air-bound system. Air and other noncondensable gases collect on the high side at the point of lowest pressure. This point may shift from the receiver to the condenser and back.

A purger is connected to the horizontal receiver on the top. It may be connected just above the liquid level in the case of vertical receivers. Location of purge line connection to condensers depends upon the type of condenser. With double-pipe condensers, correct location is near liquid-refrigerant outlet. Atmospheric condensers should have the purge line connected at the refrigerant outlet. Bleeder-type condensers should have the purge connection on the top pipe. With the horizontal shell-end-type condenser, purge lines are connected on the top at each end, while with the vertical, they are connected a few inches above the liquid refrigerant outlet. The purge connection is made at the liquid outlet in the case of evaporative condensers. Purge the receiver, then the condenser. Do not purge the condenser while it is operating.

H. L. Mitten, Jr.

677. How much does your steam cost? W. SHINN AND W. ARROTT, Operating Engineer, Albany, N. Y. Operating Engineer, 2, 7: 28-9. July, 1949.

The cost of steam, electricity, heat and other services from the power plant is important to the engineer, manager and the company's customers. Cost records show the cost of operation. Figuring costs is easy when dollars are substituted for make-up water, gal. of fuel oil or lb. of steam. An example of cost records and calculations is presented.

H. L. Mitten, Jr.

678. Die wärmepumpe in der milchindustrie. (The heating pump in the dairy industry.) English summary. K. H. SUTTOR. Die Milchwissenschaft, 3, 11: 340-344. Nov., 1948.

The discussion is concerned with the usefulness of installing a heating pump in dairy plants in order to utilize the waste heat resulting from such operations as cooling of heated milk, etc. A heating pump may prove to be economical only in plants where the cost of fuel is high as compared with electricity. Other items to consider are: (a) the discontinuous supply of waste heat throughout the day, (b) the immediate utilization of waste heat or the need for storing it for later use, and (c) the seasonal use of waste heat.

I. Peters

679. Der trennvorgang in der schleudertrommel. (The process of separation in a separator bowl.) English summary. W. WILSMANN. Die Milchwissenschaft, 3, 10: 302-309; 3, 11: 327-334; 3, 12: 366-371. Oct., Nov., Dec., 1948.

This is a theoretical discussion of the separator bowl. Calculations and diagrams dealing with the principles of efficient separation and clarification of milk are presented.

I. Peters

680. Proper air filter maintenance pays industry in good results. B. G. EVANS, Eli Lilly and Co., Indianapolis, Ind. Heating, Piping Air Conditioning, 21, 7: 86-8. July, 1949.

If filters are not cleaned or replaced when dirty, they may impede air flow and may allow dirt to be carried through because of high velocities in sections of the filter.

Some filters work better for a given application than others. A wire mesh cleanable filter is used for filtering the air supplied a building. This type filter removes enough impurities to make the air tolerable for ventilation. It has a large dust holding capacity. It may be cleaned with a solution such as kerosene by dipping and setting up to dry.

Where high purity air supply is needed, but the installation of an electrostatic filter is not justified, the author uses a filter consisting of multiple layers of paper lapped over a serrated frame. Sprayed with oil, this filter has a high efficiency of dust collection. Because of low dust holding capacity, it must be changed frequently. The electrical precipitation filter is used in applications with rigid air filtration requirements. Stationary cell-type filters are cleaned by directing a hot jet of water at 200 to 210° F. against the ionizing wires and plates.

Traveling plate electrostatic filters are used where high efficiency and large dust holding capacity are required. The plates are built into a continuous screen which rotates and dips into an oil bath for continuous cleaning.

Maintenance of filters is of prime importance for efficient removal of impurities and proper ventilation.

H. L. Mitten, Jr.

81. Design standards for grade A plant producer farm dairies. G. L. NELSON, Okla. A. and M. College, Stillwater. *Agr. Eng.*, 30, 6: 271-273. June, 1949.

Considerations leading to the design standards presented are those which have been developed cooperatively by the Oklahoma A. and M. College, Oklahoma State Health Department, and 19 county and city health departments.

The building described is congruent with the U. S. Milk Ordinance and Code; it contains a milking room, a feed room and a milk room. Herd shelter is to be provided elsewhere. There should be a feed alley and litter alley in the milking room; these will increase wall and floor requirements but decrease the work travel tremendously. The milking room should be of such size as to permit controlled grain feeding. Fifteen min. feeding time is a reasonable time. Where a two-unit milker is used, and 5 min. is allotted for preparing and milking each pair, a minimum of 6 stanchions is needed.

Proper drainage of the milking room wastes depends upon the natural drainage of the site. Milk room wastes should not be permitted to flow through the milking room.

Mangers should be 12 in. higher than stall platforms. Milk rooms may be ventilated by vertical flue ventilators. Design data which specify the ventilation rate are not known. In the milking room a 24-in. diameter flue will remove moisture at the rate produced by 6 cows if the outside temperature is 25° F. with relative humidity of 50% and air velocity is 5 mph. and the inside temperature is 35° F. with 75% relative humidity. Several designs and some comparisons are illustrated.

H. L. Mitten, Jr.

682. Calculations for milkhouse heating purposes. C. P. WAGNER AND M. NABBEN, Northern States Power Co., Minneapolis, Minn. *Agr. Eng.*, 30, 6: 294-296. June, 1949.

Milkhouse heating is desirable to protect the water system, to remove ice from the floor, to prevent condensation on walls and to provide for personal comfort of workers. A controlled temperature of 40° F. gives milkhouse protection. Milkhouses in Minnesota of the same size, heated to 40° F., require from 2000 to 8000 kw-hr./season, depending upon the insulation values and degrees of infiltration. A Minnesota milkhouse that can be heated to 40° F. by 2000 kw-hr. is calculated to require 3200 kw-hr. for heating to 50° F.

Heat requirements of a building may be reduced by locating one or more sides against another building, serving through connecting vestibule, insulating and reducing infiltration. New

milkhouse location plans should be made considering heating. In the Minneapolis area the annual value of good insulation and close fitting storm door and windows is \$40 to \$80 with electricity at 2¢/kw-hr. and heating to 40° F.

Heat removed from milk may be used to heat the milkhouse, for the ordinary mechanical refrigerator acts as a heat pump. The radiator (condenser) has an output of approximately 2.3 kw-hr. (7850 BTU) for each 10 gal. can of milk handled (milk temperature reduced from 95° F. to 40° F.). The milk cooler may be supplied with well water to supplement the milk as a heat supply. A milk cooler compressor of 0.75 hp. or larger would be required for this application. The use of the milk cooler as a heat pump is still in the experimental stage.

Additional methods of applying heat are direct radiation, storage water heater, radiant heat (wall or floor), heat pads and heat lamps. Fan-type, direct radiation heaters seem to be the best units for supplementary heating.

The well-insulated milkhouse of 1000-12000 ft.³ can be expected to require 0.75 kw-hr. of direct heat/degree-day. The same milkhouse may be heated with the milk cooler operating as a heat pump for 0.25 kw-hr. or less/degree-day. This includes energy used to cool the milk.

H. L. Mitten, Jr.

683. Are your trap discharge lines right size? H. G. EBERT, Yarnall-Waring Co., Philadelphia, Pa. *Operating Engineer*, 2, 6: 26-7. June, 1949.

Trap discharge-line size is often as important as a properly sized trap. Charts are presented which give % of flash when condensate enters a trap, equivalent length of pipe to allow for the fittings in the line, and discharge-pipe diameter. Discharge lines sized by these charts will help traps drain equipment rapidly.

H. L. Mitten, Jr.

684. Safety is big business here—\$640.00 saved in two years. W. ARROTT, Operating Engineer, Albany, N. Y. *Operating Engineer*, 2, 7: 20-22. July, 1949.

The safety program of the 74th St., New York power plant is described. Step one in any program is education. The men who run the plants should be responsible for safety and for teaching safety to the workers. Classes were built around films of the National Safety Council. The second step is a safety program. Competition builds enthusiasm among workers. The third step is safety follow-up. After each accident, the follow-up determines the cause, and steps are taken to prevent recurrences. Safety saves working time and boosts morale.

H. L. Mitten, Jr.

685. Low down on wearing rings. I. J. KARASIK AND R. CARTER, Worthington Pump and Machinery Corp., Harrison, N. J. *Operating Engineer*, 2, 7: 38-39. July, 1949.

Centrifugal pumps have running joints between their impeller and casing. Clearance in these joints is usually between removable wearing rings. The different types of rings are presented by diagram and discussed as to construction, location, mounting and clearances. H. L. Mitten, Jr.

Also see abs. no. 713.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

686. The ice cream market in the home. Anonymous. *Ice Cream Trade J.*, 45, 7: 34, 36, 77-79. July, 1949.

According to a survey just made by the U.S.D.A., the average family of 3.42 persons in 68 cities spends 42.5¢ for ice cream for home consumption. The amount spent ranged from 17¢/wk. for families whose income was under \$1,000/yr. to 73.2¢ for families with incomes of \$7,500 or over. The average family spent \$25.57 weekly for food, 1.66% of which was spent for ice cream.

Assuming that the average per capita consumption in 1948 was 16.23 qt./person, the average family would have consumed 55.5 qt./yr., or \$38.50 worth at 70¢/qt. The government study indicated that about half of the ice cream produced is going into the home, the other half being consumed at the point-of-sale. The amount of money spent for ice cream ranked high when compared with the amount spent for other dairy products. The average family spent \$2.21/wk. for fluid milk, 65.6¢ for butter, 52.6¢ for all types of cheese and 42.5¢ for ice cream. W. H. Martin

687. What the industry should do about costly practices that are unsound and unethical. J. H. MEEHAN, Phila. Dairy Products Co. *Ice Cream Trade J.*, 45, 6: 46, 48, 99. June, 1949.

The unsound and unethical practices which have beset the ice cream industry are unfair to the producers as well as the dealers. Management, which is at fault, should stop this cutthroat competition and train their salesmen to sell ice cream on the basis of intelligent constructive sales instead of underselling competitors and bribing dealers with cabinets and neon signs. Salesmen should stress selling more gallonage to their customers instead of fighting for other companies' customers; thus the gallonage sales would increase, the cost of ice cream decrease, and better feeling would result among members of the association.

W. H. Martin

688. Snowballs in July. Anonymous. *Ice Cream Trade J.*, 45, 6: 44. June, 1949.

An unexpected but very welcome advertisement of ice cream will reach 43 million readers through the efforts of Durkee Famous Foods campaign to acquaint the public with Coconut Snowballs. These "snowballs" are ice cream covered with chocolate and topped with coconut. They are sold in the Stork Club and other eating places for as high as \$1.50, but are really very inexpensive so they should appeal to the fountain and home consumers. This extensive advertisement will start just as the June Dairy month campaign ends, thus keeping public attention focused on ice cream. W. H. Martin

689. While the show goes on, there's gallonage in theatres. T. E. HEIDENREICH, JR. *Ice Cream Trade J.*, 45, 5: 46, 48, 113-114. May, 1949.

Theatre operators, hunting a new source of revenue to support their dropping ticket sales, have provided a comparatively new "dry stop" for ice cream manufacturers. In a recent *Motion Picture Herald* survey of 16,880 theatres, 20% or 3,201 were selling ice cream, while 89% sold candy. The gallonage demand is as high, if not higher, in the winter months as in summer, making a year-round market with only a slight slump in Apr.-May and Sept.-Oct. In 1948, theatre gallonage sales increased over the previous year's sale and so far in 1949 the increase has been substantial.

The few retail difficulties such as proper storage cabinets, an ice cream product which will leave a minimum of litter in the theatre, and the method of selling either by peddling in the aisles or selling from the lobby stands are being considered and overcome. This "dry stop" adds up to some important gallonage for the industry in years to come. W. H. Martin

690. High's converts to self-service. H. HAUG. *Ice Cream Trade J.*, 45, 5: 52-53, 108. May, 1949.

High's Dairy Products Co. of Washington, D. C., are converting most of their 67 stores from counter-type to self-service after experimenting with a store which moved from 12th to 3rd place in sales volume after self-service had been installed.

Though the equipment for self-service costs about \$6,000 as compared to \$1,800 for counter sales equipment and the electricity bills run 3 and 4 times higher, the greater volume of business more than pays for the change. There was a 50% increase in milk sales when the open sales cases were substituted for the glass door reach-in type cases, and the ratio of hand-dipped and packaged

ice cream sales changed from 3 hand dipped quarts to 1 packaged quart previously and is now 4 packaged quarts to 1 hand dipped.

The same number of attendants is maintained but they handle a much larger amount of business. Long periods of waiting have been eliminated, inventories are easier and there is no additional administrative problem. W. H. Martin

691. A legislative commission looks at the home delivery of milk. Anonymous. *Am. Milk Rev.*, 11, 6: 36-39, 41. June, 1949.

This article is a summary of a study in New York City conducted under the direction of Dr. E. C. Young, Dean of the Graduate School at Purdue Univ., at the request of a temporary commission on agriculture created by the legislature of the State of New York in 1945. The report was submitted in March, 1949.

The duties of a home delivery routeman were stated as follows: (a) get vehicle and return it to garage, (b) load and unload, (c) drive to route and return, (d) deliver, (e) collect, (f) record sales and collections and (g) settle accounts and make out order. The average routeman spent 60% of his time for actual service to the customer and 40% for bookkeeping, loading, and unloading, credits, and collections. Wide variations in delivery costs were noted and attributed to differences in consumer dwelling and buying habits, standards of customer service, number of units delivered/customer and responsibilities of the routeman. The cost of the average customer call was 18.5¢ with an average of 2.86 qts./customer, resulting in a cost/qt. of 6.5¢. When the customers lived primarily in row houses the cost/call was 8.6¢. When customers lived in separated homes the cost was 12.4¢/call. Since no price reductions were offered for quantity purchases by multiple route companies, no incentive existed for revising buying habits of customers. Home delivery of milk offers more service to consumers than any other food product. D. J. Hankinson

692. Prevailing wages paid to milk routemen. C. LEWIS, Univ. of Missouri, Columbia. *Milk Dealer*, 38, 8: 46-47, 138. May, 1949.

A survey of prevailing wages paid to milk routemen gave enough information to conclude that the wages, including commissions, paid these workers range from \$59.37 to \$87.37, with an average of \$66.66/wk. Commissions averaged between 5% and 8% on wholesale routes and a little more on retail routes. The wholesale price of pasteurized milk ranged from 15 to 24¢/qt. with an average price of 17.99¢/qt. The retail price ranged from 17 to 24¢ with an average price of 19.85¢. Homogenized milk had the same price range as re-

tail pasteurized milk but averaged 20.69¢/qt. Wage cost/qt. varied between 1.25¢ to 11¢ with an average of 3.66¢/qt. on wholesale and 3.869¢/qt. on retail. Tables are presented showing distribution of milk prices for Feb., 1949, wage groups disposition, and the weekly wages paid routemen, and prices for retail and wholesale bottle milk for Feb., 1949. C. J. Babcock

693. Selling salesmen to sell. R. G. PFAT, Silverwood Dairies, Ltd., London, Ontario. *Am. Milk Rev.*, 11, 7: 2-4. July, 1949.

This article points out 6 factors which make for successful selling: (a) selection of personnel, (b) training, (c) planned selling, (d) incentive, (e) recognition, and (f) supervision. The use of all factors is summed up under one term—leadership. D. J. Hankinson

694. Where do you break even? M. J. KLUGER. *Am. Milk Rev.*, 11, 6: 2-4, 6. June, 1949.

"Break-even" point is a term used by accountants to indicate the sales volume just necessary to pay for expenses which do not vary with sales volume in addition to the expenses which vary with sales. It is useful as a management tool to maintain sufficient volume to insure profit. This is especially important in the milk industry where the margin of profit is narrow. The break-even point varies between plants and from month to month. It is pointed out that a reduction in volume of sales may result in a net loss to the plant because certain fixed expenses do not change with volume of business. The break-even point can be represented in chart form to give a visual guide for conduct of business. D. J. Hankinson

695. Allocation of costs in multiple products plants. L. C. THOMSEN, University of Wis. *Natl. Butter Cheese J.*, 40, 6: 28-29, 56-58. June, 1949.

In multiple products plants it is difficult to allocate costs of such secondary operations as quality control, and receiving, separating and administrative costs. This paper discusses various methods of allocating these secondary costs to the cost of the major product. A dairy plant must have a sound accounting system before any method of cost allocation is practical. Adoption of standard accounting procedures will result in greater permanency for the accounting system. This will make possible long term comparisons of costs, sales, etc. H. E. Calbert

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

696. The value of urea in protein supplements for cattle and sheep. J. S. DINNING, H. M.

BRIGGS AND W. D. GALLUP, Oklahoma Agr. Expt. Station, Stillwater. *J. Animal Sci.*, 8, 1: 24-34. Feb., 1949.

Nitrogen balance studies were conducted on 2 yr. old steers and growing wether lambs on maintenance wintering, and fattening rations in which urea was included in varying amounts from 9 to 50% of the total N of the ration. The steers refused to consume all of the rations in which urea furnished 50% of the N, but no difficulty was experienced in getting the lambs to eat rations containing that level of N.

The amount of N retention for both steers and lambs was increased when additional N was furnished by urea. The addition of urea did not increase fecal N but caused an increase in the total N of the urine, largely in NH_3 and urea. Both steers and lambs reacted alike in this respect. Lambs apparently were more efficient in the utilization of urea. Total N retained was about the same in rations in which urea furnished 50% of the N, as in those in which it supplied 25%. Urea increased the apparent digestibility of protein but had no effect on the digestibility of other nutrients. F. C. Fountaine

697. The influence of soybean oil meal upon roughage digestion in cattle. W. BURROUGHS AND P. GERLAUGH, Ohio Agr. Expt. Station, Columbus. *J. Animal Sci.*, 8, 1: 3-8. Feb., 1949.

The digestion coefficients of dry matter in corn-cobs and timothy hay were determined by feeding fattening cattle rations to steers. Two rations, 1 with and 1 without corn-cobs, were fed both at an 8% and at a 15% protein level. Soybean oil meal was substituted for corn to change the protein content of the rations. The digestion of dry matter in corn-cobs was increased by 14% and that of timothy hay by 17% by the addition of soybean meal to the ration. F. C. Fountaine

698. Further observations on the effect of protein upon roughage digestion in cattle. W. BURROUGHS, P. GERLAUGH, B. H. EDGINGTON AND R. M. BETHKE, Ohio Agr. Expt. Station, Columbus. *J. Animal Sci.*, 8, 1: 9-18. Feb., 1949.

Five series of digestion trials with beef steers were conducted to determine the effect of protein on dry matter digestion of corn-cobs or clover hay fed as sole roughages. In 2 series with corn-cobs and 1 with clover hay, the protein content of the ration was varied by substituting dried skim-milk for mineralized starch. In one series dried skim-milk was added in varying amounts directly to the corn-cobs and in the final series dried skim-milk was added in different amounts to a constant mixture of starch and corn-cobs.

In every instance when starch was fed as part

of the ration, the addition of dried skim-milk improved the apparent digestibility of the roughage. Adding dried skim-milk to a ration in which no starch was present had little effect on the digestion of the dry matter in corn-cobs, even though the total ration contained as little as 4% total protein. When starch was present the minimum level of protein for efficient digestion seemed to be between 8 and 12%. F. C. Fountaine

699. The growth of dairy heifers raised chiefly on roughages. O. T. STALLCUP, H. A. HERMAN AND A. C. RAGSDALE. *Mo. Agr. Expt. Sta. Bull.* 523. 1949.

Twenty-eight Holstein heifers nursed their dams 3-4 d., received whole milk to 6-7 mo., with lespedeza hay, alfalfa hay, sorgo silage, limited concentrates at 3 levels, and pasture in season to 24 mo. Others received limited milk to 8 wk., an 18% protein calf starter, lespedeza hay and pasture in season. Calves were encouraged to take roughage and concentrates early. Normal growth was obtained where about 55% of the crude protein and digestible nutrients were obtained from pasture. Dairy heifers of normal weight and height may be reared from 6 to 24 mo. of age on not more than 900 lb. concentrates, if quality roughage and pasture are provided in abundance. Feed consumptions and body measurements are given. R. B. Becker

700. The metabolism of niacin in ruminants (sheep, goats and calves). P. B. PEARSON, W. A. PERLZWEIG AND F. ROSEN, A. & M. College of Texas, and Duke Univ. School of Medicine. *Arch. Biochem.*, 22, 2: 191-94. June, 1949.

The urinary excretion of nicotinic acid, N-methylnicotinamide (NMN) and its pyridone, N-methyl-6-pyridone-3-carboxylamide was determined for calves (about 6 wk. old) and mature goats and sheep on a normal diet and following the ingestion or parenteral administration of 2 g. nicotinamide daily for 3 consecutive d. Between 14 and 19% of the niacin ingested by calves and sheep is excreted by the renal pathway as non-methylated nicotinic acid derivatives. Goats excreted only 6.4% when nicotinamide was ingested, but 23% when administered subcutaneously. A small but insignificant increase in the amount of NMN excreted was observed for all three species, thus exhibiting a behavior similar to that reported for the rabbit, guinea pig and horse. On a normal diet neither the calf, goat nor sheep excreted measurable amounts of pyridone. The results obtained with nicotinamide administration further reveal that pyridone is not an end product of niacin metabolism in the calf, and that it is of minor quantitative importance in the goat and

sheep. The observations do not preclude the possibility of both methylated derivatives being intermediates in the metabolism of niacin by herbivora, since the horse and guinea pig have been shown to destroy NMN, while the rabbit oxidizes a considerable quantity of it to the pyridone. The enzyme capable of oxidizing NMN has been found in rabbit liver but not in sheep liver.

H. J. Peppler

701. Studies of the effect of phosphate fertilization on the composition and nutritive value of certain forages for sheep. G. MATRONE, R. L. LOVVORN, W. J. PETERSON, F. H. SMITH AND J. A. WEYBREW. *J. Animal Sci.*, **8**, 1: 41-51. Feb., 1949.

Phosphate fertilization of Bladen silt loam of the North Carolina Coastal Plain had no effect on the chemical composition of soybean hay grown on it. When measured by gain in wt. and apparent digestibility there was no significant difference in the feeding value for lambs of a ration of soybean hay and raw soybeans grown on phosphate fertilized plots and one grown on check plots.

In the 2nd yr., with cerclose as a concentrate, soybean hay grown on phosphated soil gave significantly greater gains in lamb and had a higher apparent digestibility than soybean hay grown on unphosphated plots. The authors suggest that general conclusions should await results of further investigations.

F. C. Fountaine

702. Phosphatic animal-feed supplement. Laboratory and pilot plant production. G. L. BRIDGER, J. W. MOORE AND H. M. McLEOD, JR. Tenn. Valley Authority, Wilson Dam, Ala. Animal feeding tests. D. E. Williams, F. L. McLeod, E. Morrell and H. Patrick, Univ. of Tenn. Agr. Expt. Sta., Knoxville, in coop. with Tenn. Valley Authority. *Indus. Eng. Chem.*, **41**, 7: 1391-1400. July, 1949.

A waste material composed chiefly of iron and phosphorus in a form unavailable to plants and animals was converted to a limestone-ferrophosphorus product. In the new form about 3/4ths of the phosphorus was available for phosphorus retention to experimental animals (rats and chicks), as compared with readily available phosphorus in a salt mixture. This partial unavailability was overcome by feeding the product at increased levels. This material may be a useful phosphatic feed supplement if it can be produced cheaply.

B. H. Webb

703. The calcium, magnesium and potassium contents of the serum of ewes fed high levels of potassium. P. B. PEARSON, J. A. GRAY AND R. REISER, A. & M. College of Texas, College Station. *J. Animal Sci.*, **8**, 1: 52-56. Feb., 1949.

Potassium bicarbonate included as approximately 5% of a ration of alfalfa hay and grain had no significant effect on the amount of calcium, magnesium and potassium in the blood serum of mature ewes.

F. C. Fountaine

704. The influence of tocopherols upon the mammary and placental transfer of Vitamin A in the sheep, goat and pig. F. WHITING, J. K. LOOSLI AND J. P. WILLMAN, Cornell Univ., Ithaca, N. Y. *J. Animal Sci.*, **8**, 1: 35-40. Feb., 1949.

Three prepartal rations furnishing respectively 12,000 I. U. Vitamin A, 80 mg. tocopherol, and 12,000 I. U. Vitamin A plus 80 mg. tocopherol daily were compared to basal rations for ewes, goats and sows.

Supplementing the prepartal rations with 12,000 I. U. of Vitamin A resulted in increased stores of Vitamin A in the livers of the newborn, and in the colostrum of all species studied. Addition of 80 mg. of tocopherol to the basal ration increased the liver stores of only lambs. Tocopherol supplements had no influence on the Vitamin A content of the colostrum. Combination of tocopherol and Vitamin A supplements had no effect on liver stores of the newborn or the Vitamin A content of the colostrum, as compared to rations containing only Vitamin A supplements.

F. C. Fountaine

Also see abs. no. 635, 721.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

705. A metabolic regulator in mammalian spermatozoa. H. A. LARDY, D. GHOSH AND G. W. E. PLAUT, Univ. of Wisconsin, Madison. *Science*, **109**, 2832: 365. Apr. 8, 1949.

Metabolic data, together with motility observations obtained on epididymal and ejaculated bovine spermatozoa lead to the discovery of a metabolic regulator. This substance is present in bound form in epididymal spermatozoa and apparently is liberated into the seminal fluid as a water-soluble conjugate from which the active form is released by mild alkaline hydrolysis. The substance also is liberated during prolonged storage of excised epididimides in the refrigerator. Aerobic fermentation by yeast is stimulated by the regulator, and this characteristic forms the basis for an assay, the details of which are to be reported elsewhere. The regulator is believed to be responsible for the high rate of respiration of ejaculated spermatozoa. The possibility is advanced that if liberation of the regulator could be prevented, or a method of counteracting its effects devised, viable spermatozoa could be preserved for longer periods.

M. Loewenstein

706. Effects of dilution on motility of bull spermatozoa and the relation between motility in high dilution and fertility. P CHENG, L. E. CASIDA AND G. R. BARRETT, Univ. of Wisconsin, Madison. *J. Animal Sci.*, 8, 1: 81-88. Feb., 1949.

Six semen samples from each of 5 bulls were diluted at 1:10, and successively from 1:100 to 1:12,800 with 0.9 NaCl and with 0.08 *M* sodium citrate diluents. There was a progressive decrease in % of motile spermatozoa from low to high dilutions. Motility was not restored in spermatozoa reconcentrated by centrifuging. The addition of egg yolk to citrate buffer markedly increased the motility of spermatozoa in dilutions from 1:100 to 1:12,800. There was no significant correlation between motility and fertility when measured within bulls.

F. C. Fountaine

707. Evidence of an inherited seminal character associated with infertility of Friesian bulls. J. L. HANCOCK, Wellcome Veterinary Research Station, Frant, Sussex. *Vet. Record*, 61, 22: 308-309. May 28, 1949.

A morphological abnormality of the spermatozoa of 7 closely related Holstein-Friesian bulls, all of which had a very poor breeding record, is described. This abnormality appeared only after staining, and photomicrographs are shown of affected spermatozoa stained with iron haematoxylin where the abnormality appeared as a deeply stained area at the anterior pole of the head. Other semen characteristics, including motility ratings, density, viability at 4° C., and methylene blue reduction time, all were within the normal range on these bulls. The average percentage of affected spermatozoa ranged from 79 to 96%, and the bulls were used in 6 different herds on 108 females, none of which became pregnant. When these females were mated to known fertile bulls, clinical histories indicated they were of normal fertility.

R. P. Niedermeier

708. An apparatus for the extraction of fertilized eggs from the living cow. L. E. ROWSON AND D. F. DOWLING. *Vet. Record*, 61, 15: 191. April 9, 1949.

An apparatus that enables one to wash the ova from the uterine horn with a minimum amount of irrigating medium is described in detail. It consists of a solid rubber tube about 30 in. long with 3 channels. Two of the channels act as a two-way catheter with 1 opening near the tip and the 2nd a few inches back. The 3rd channel opens into a 1 in. latex collar vulcanized to the tube just behind the 2nd opening of the two-way system. This collar can be inflated with air after the tube is in position and seals off the tip of the uterine horn. The technique of inserting this irrigating

tube with the aid of a steel stilette, withdrawn after insertion, also is described. Epidural anaesthesia is suggested as being of help in controlling the operation. The name of a London firm now manufacturing this apparatus is given.

R. P. Niedermeier

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

709. Instrumentation for animal shelter research. C. F. KELLY, T. E. BAND AND C. LORENZEN, JR., U. S. D. A. *Agr. Eng.*, 30, 6: 297-304. June, 1949.

Research in animal shelters many times involves measurement of heat flow. Temperature and heat flow are so closely related that instruments for measuring one usually are useful for measuring the other. The estimation of heat loss rate by convection requires the knowledge of dry-bulb temperature of air and rate of air flow. Rate of heat loss by radiation involves the temperature of surfaces or the direct measurement of emission. Wet-bulb temperature of air is required in the estimation of total heat loss and moisture removal through ventilating systems. Instruments available are radiometers for measuring total solar and sky radiation and intensity of radiation from a surface; globe thermometer and Kata thermometer for measuring the combined effects of temperature, radiation and air flow; touch thermocouple for measuring the wet-bulb depression; and potentiometers for measuring and recording the thermoelectric effect of the preceding instruments.

The thermocouple is used in making surface temperature measurements. The diameter of the wire should be small; 30 or 36 gauge wire with a butt-welded junction is satisfactory. For surface temperature of swine and cattle a touch thermocouple has been used.

The wet-bulb temperature of air can be measured satisfactorily with a thermoelectric psychrometer which uses a single wet and dry junction of 36 gauge copper and constantan. Dust from feed and floor make frequent changing of wicks necessary. Facial tissue and cigarette paper are satisfactory substitutes for absorbent cotton wicks and are easier to change.

Small heat flow meters fitted with handles can be used to measure heat flow by radiation and convection from walls, floors, ground or animals. The instrument may be cemented, screwed or taped to the surface being tested. When fitted with a suitable handle it can be held against the animal. With animals, the time the instrument is held against the animal should be sufficient to allow heat transfer rate to reach a steady state, but not so long as to materially affect the animal's subcutaneous circulation.

The flat plate radiometer is used for measuring the heat load on animals and structures from both the sky and ground, and for comparing the efficiency of shades in cutting off solar radiation. A device for calibrating small radiometers is described and a formula for determining the calibrating factor is given.

Potentiometers for general purpose work should be calibrated in millivolts and need not have an automatic cold junction compensator.

H. L. Mitten, Jr.

710. Milking barn. G. J. AND D. R. POLIVKA. U. S. Patent 2,472,122. 9 claims. June 7, 1949. Official Gaz. U. S. Pat. Office, **623**, 1: 128. 1949.

To facilitate machine milking of cows, the stalls of this milking barn are raised a few feet above the floor level. The cows enter the stalls from raised aisles. To conserve space and to provide convenience the stalls are set at an angle to the aisles.

R. Whitaker

711. Teat cup. H. O. LINDREN (assignor to Aktiebolaget Separator Corp.). U. S. Patent 2,473,379. 1 claim. June 14, 1949. Official Gaz. U. S. Pat. Office, **623**, 2: 578. 1949.

A teat cup for milking machines consisting of a rigid shell containing a flexible inner liner which is caused to extend and contract by a pulsing source of vacuum applied through a tube separate from the milk collecting tube.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

712. Year 'round specialty. Anonymous. Ice Cream Trade J., **45**, 7: 44, 95. July, 1949.

Cake for the "ice cream 'n cake" roll is received in sheets 24 in. long from the Newly Weds Baking Co. Chicago plant. In the plant, pans are lined with sheets of white waxed paper $19 \times 24\frac{1}{2}$ in. and the cake inverted is placed in the pan on top of the paper. Ice cream from a continuous freezer is spread over the cake by means of a special spreading device; the pans pass on a conveyor to a table where the roll is formed; a stainless steel miter box and saw is used to cut the 24 in. roll into 6 uniform sections. Eight sections are packed into a carton. The Standard Drug. Co. of Cleveland, O., markets this item through its 53 outlets at 39¢ for a 4" roll. Different flavor combinations are made, including vanilla ice cream and chocolate cake, and vanilla and strawberry ice cream with vanilla cake.

W. H. Martin

713. A guide to cabinet sizes. Anonymous. Ice Cream Trade J., **45**, 7: 58. July, 1949.

A special chart has been prepared by the Ice Cream and Cabinet Section of the Air Conditioning and Refrigeration Machinery Association. It gives the dimensions of the 1949 conventional type cabinets manufactured by its members.

W. H. Martin

714. Golden State markets "Redi-Serv" for single-service at the dealer fountain. Anonymous. Ice Cream Trade J., **45**, 6: 42-43, 88. June, 1949.

In a search for controlled ice cream portions, the San Francisco Golden State Co., Ltd., started producing last May the factory made single-service ice cream item, Golden State "Redi-Serv." It is extruded from a freezer at 18° F. It is deposited in a solid mass and touches only the bottom of the cup, not the sides. This necessitates constant low temperature. The portions may be adjusted by the delivery tube. At present the size is 2.4 fluid oz. These cups are packed in 4 half-dozen layers separated by wax paper, sealed and turned over so the cups are upside down and the ice cream protected at all times when being dispensed.

The advantages of this "Redi-Serv" are many. The soufflé cup which one pushes on the bottom to release the ice cream is faster, more sanitary, has no waste, fits standard cones and other dispenser equipment and gives the dealer controlled portions. This cup is sold to dealers for about 3.5 cents, making it possible for him to figure exactly his costs and is promoting a 10 cent sundae. Consumer reaction to texture and flavor has been excellent.

W. H. Martin

715. Shifts in gallonage. C. E. FRENCH, Purdue Univ., LaFayette, Ind. Ice Cream Trade J., **45**, 7: 38, 39. July, 1949.

Since 1925 Pennsylvania and New York State have occupied the first 2 positions in total ice cream production. California, Illinois and Ohio are next 3 ranking states. Greatest gains have been made by North Carolina and Tennessee, which have moved up to 14th and 15th places from 24th and 26th places, respectively. Other southern states have moved up substantially.

W. H. Martin

716. A study of sales and profits in malteds at the retail level. Anonymous. Ice Cream Trade J., **45**, 6: 66, 79. June, 1949.

The *Fountain Service Magazine* made a recent survey throughout the country which showed that though the Midwest makes a richer malt, the East and West sell more and profit more due to their lower prices and sound merchandising techniques. This survey also shows flavor preference

the country over the same, with chocolate rated first with 78%, vanilla next with 11%, strawberry with 6.6%, pineapple polling 3.1%, and all other flavors accounting for the remaining 1.3%.

W. H. Martin

717. Ice cream volume forges ahead. Anonymous. *Ice Cream Trade J.*, 45, 7: 52, 91. July, 1949.

The volume of ice cream made in May, 1949, was 55,770,000 gal., representing an increase of 3% over May, 1948. Pennsylvania and New York were 8 and 10% ahead of last year. Massachusetts, up 12%, Indiana 9%, Michigan 11%, Washington State up 31%, and Oregon up 24%, were other states showing substantial increases. Production for the first 5 mo. was 204,450,000 gal. or 3% below 1948.

W. H. Martin

Also see abs. no. 674, 686, 687, 688, 689, 690.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

718. Tank truck hauling of milk from farm to plant. D. C. LIGHTNER, Creamery Package Mfg. Co., Chicago, Ill. *Milk Dealer*, 38, 8: 42-43, 98-100. May, 1949.

Tank truck hauling of milk from farm to plant is practiced on the West Coast, especially in California, where the milking herds are large. The Bryant and Chapman Dairy of Hartford, Conn., is successfully using the system with smaller herds. Before buying a tanker the processor should determine the number of producers on or near a good highway, accessibility of the milk-house for loading the tanker and to the highway, electric power for refrigeration, and whether or not the quality of the milk produced on a farm is sufficiently uniform so that it is safe to mix it with other milk. Methods of cooling and storing the milk on the farm are discussed. It is believed that this method of transporting milk from the farm will grow and spread and that it may, in time, revolutionize milk production by eliminating the small inefficient producer. The processors apparently like it because it gives them control of the quality of the milk and producers like it because they have control over weighing and the taking of samples.

C. J. Babcock

719. Canned fresh milk is a fact. W. RUDOLPH. *Am. Milk Rev.*, 11, 5: 2, 3, 53. May, 1949.

A method for canning milk immediately after it is drawn from the cow and pasteurized is described as the Stambaugh-Graves method, named for the 2 men who developed the process. It includes drawing the milk by vacuum (milking machines) to a glass weighing jar, from whence additional

vacuum draws it to a storage vat. The milk then is homogenized at drawing temperature, followed by preheating to 190° F. The next step is heating in a special-type exchanger to 260° F. for 19 sec., after which the milk is placed in lacquered cans. The filled cans are sterilized (no information on this process is given). Nitrogen gas is used to blanket milk that otherwise would be exposed to air. Economies in marketing milk are claimed if the pilot plant scale operation can be adapted to commercial scale production. Distribution to areas where milk is not readily available would be possible.

D. J. Hankinson

720. Ready-to-use whipped cream in cans. Anonymous. *Milk Dealer*, 38, 9: 46, 90-91. June, 1949.

The sale of whipped cream in single metal containers is producing a profit for a growing number of distributors of this product. Experiences in several markets are related. The mix is made up with cream, vegetable stabilizer, condensed skim-milk (for additional milk solids), sugar and pure vanilla for flavor. The mix is standardized to a 30% butterfat content and pasteurized the same as an ice cream mix is pasteurized. The whipped cream containers are a 12 oz. size and are filled with 7 oz. of mix. The can is then charged with a combination of nitrous oxide and carbon dioxide gas.

C. J. Babcock

Also see abs. no. 651, 652, 658, 660, 670, 672, 679, 681, 682, 690, 691, 692.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

721. Use of L-thyroxine by mouth for stimulating milk secretion in lactating cows. G. L. BAILEY, S. BARTLETT AND S. J. FOLLEY. *Nature*, 163, 4151: 800. 1949.

Daily oral dosages per cow of 25, 50, 100 and 150 mg. L-thyroxine and 1500 mg. iodo-casein resulted in a milk yield after 3 wk. of 1.6, 3.6, 5.9, 6.3 and 4.3 lb./cow/day. The heart rate increase after 3 wk. was 5.2, 8.7, 15.5, 21.5 and 11.2 beats/cow/min. The 150 mg. dosage appeared excessive as indicated by sweating of the cows after 2 wk. There is some indication that the published estimates of the thyroxine content of iodo-casein are in error or that the efficiency of oral utilization of thyroxine in iodo-casein is much lower than that of the hormone. The oral/parenteral ratio for L-thyroxine in the cow is about 16:1.

R. Whitaker

722. Observations on the effects of prepartal and postpartal estrogens and progesterone treatment on lactation in the rat. S. M. WALKER AND J. I.

MATTHEWS, Washington Univ. School of Medicine, St. Louis. *Endocrinology*, **44**, 1: 8-17. Jan., 1949.

Attempts were made to inhibit lactation in intact and in ovariectomized rats using the growth rate of nursing young as a measure of milk secretion rate. Treatment with 200 γ of estrone or diethylstilbestrol dipropionate daily begun on 2nd d. of lactation did not inhibit secretion in ovariectomized rats; 5 and 10 γ daily of estrone inhibited lactation in intact rats 10 to 12 d. after the beginning of treatment. Lactation inhibition induced by 1 mg. of diethylstilbestrol dipropionate, both in intact and ovariectomized rats, was accompanied by marked loss of weight of the mothers and was interpreted as a toxic effect. Prepartal injection of estrone did not prevent the initiation of lactation in either intact (25 γ and 100 γ) or ovariectomized (25 γ) rats; it did produce a delayed depression in intact rats. Daily prepartal treatment with progesterone, 2.5 mg. and 5 mg., neither prevented nor inhibited lactation. Simultaneous injection of estrone and progesterone, begun either prepartally or postpartally, did not prevent lactation but it did inhibit established lactation after 10 to 12 d. of treatment. The stimulation of mammary growth by the combined action of estrone and progesterone was thought to play a role in the inhibition of milk secretion in the rat.

R. P. Reece

723. Effects of restricted feed intake in intact and ovariectomized rats on pituitary lactogen and gonadotrophin. J. MEITES AND J. O. REED, Michigan State College, East Lansing. *Proc. Soc. Exptl. Biol. Med.*, **70**, 3: 513-516. Mar., 1949.

Forty-eight intact female rats (200 g.) and 35 rats (230 g.) which had been ovariectomized about a month previous were used in the study. They were divided into 5 groups: *ad libitum*, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{3}$ and no-feed regimes. The unfed groups were killed at the end of 7 d. and all other groups at the end of 14 d. Assays showed a pituitary lactogen content in intact and ovariectomized rats on the $\frac{1}{2}$, $\frac{1}{3}$, and no-feed regimes below that of the $\frac{3}{4}$ and full-fed controls. No change was observed in the gonadotrophic content of the pituitaries of either the intact or ovariectomized rats, regardless of the level of feed intake. In the intact, but not in the ovariectomized rats, restricted feed intake caused a marked reduction in the weights of the pituitary, thyroid and adrenals, except that on the no-feed regime adrenal weight was increased in both groups.

R. P. Reece

724. The effects of estrogen on mammary structure of adrenalectomized and thiouracil treated castrate rats. R. F. JOHNSTON AND J. F. SMITH-COBS, Michigan State College, East Lansing. *Endocrinology*, **43**, 4: 193-201. Oct., 1948.

Seventy-two albino rats were castrated at 3 wk. of age and placed into 8 groups. They received, *ad libitum*, a ration for laboratory animals and were maintained at constant temperature (76° F.) and humidity. Adrenalectomy, thiouracil treatment and estrogen treatment either alone or in various combinations were carried out when the rats were of similar age. Comparison on the basis of estrogen treatment was made on litter mates. Adrenalectomized rats received drinking water that contained 1% NaCl. Thiouracil was fed at the rate of 0.1% in the feed for 45 d. The estrogen diethylstilbestrol was injected subcutaneously at the rate of 10 γ daily during the last 10 d. of the experimental period. The rats were sacrificed the day after the last injection. The right abdominal mammary gland was removed, stained in toto with Harris's hematoxylin, and examined. At autopsy examination was made for the presence of cortical tissue. Thiouracil feeding resulted in a shortened and thickened mammary duct system. Estrogen and thiouracil treatment produced a mammary gland showing shortened and thickened ducts and considerable lobule-alveolar development. Rats that had been adrenalectomized for 55 d. had long atrophic ducts. Estrogen treatment and adrenalectomy produced mammary glands that were extensive in area and lobule-alveolar development greater than that of estrogen-treated, castrated rats. Adrenalectomy and thiouracil feeding resulted in mammary glands with a very short atrophic duct system. The addition of estrogen resulted in a mammary gland with a short thick duct system and considerably more lobule-alveolar development than any other group.

R. P. Reece

725. Changes in the distribution and concentration of alkaline phosphatases in tissues of the rat after hypophysectomy or gonadectomy, and after replacement therapy. E. W. DEMPSEY, R. O. GREP AND H. W. DEANE, Harvard Univ., Cambridge, Mass. *Endocrinology*, **44**, 1: 88-103. Jan., 1949.

It was shown that after either hypophysectomy or gonadectomy, phosphatase persisted in the mammary glands of rats, although in reduced amounts. It was thought, therefore, that the enzyme activity of the mammary gland does not depend completely upon the hormonal stimuli emanating from the hypophysis or ovaries.

R. P. Reece

Also see abs. no. 704, 733.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

726. Comparative nutritive value of butter and

vegetable fats under conditions of low environmental temperature. B. H. ERSHOFF, J. N. PAGONES AND H. J. DEUEL, JR., Emory W. Thurston Lab. and Univ. of Southern California School of Medicine, Los Angeles. *Proc. Soc. Exptl. Biol. Med.*, 70, 2: 287-290. Feb., 1949.

The nutritive value of fats was determined under the stress of low environmental temperature. Immature female rats were raised to maturity in a large walk-in refrigerator at a temperature of $2 \pm 1.5^\circ \text{C}$. and under standard laboratory conditions at an average temperature of $21 \pm 2^\circ \text{C}$. Purified rations were fed differing only in source of fat. The fats employed were cottonseed oil, corn oil, margarine fat and butterfat. Body weight gain was significantly reduced in all rats under cold room conditions. The gains in body weight on the various diets were not significantly different, either under cold room or room temperature conditions. R. P. Reece

Also see abs. no. 666.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

727. The relative growth of the thyroid gland in the bovine fetus. C. W. NICHOLS, JR., I. L. CHAIKOFF AND J. WOLFF, Univ. of California Medical School, Berkeley. *Endocrinology*, 44, 6: 502-509. June, 1949.

Thyroid gland growth, in relation to body weight, body length and age, was investigated in the bovine fetus from 62 d. to term. Fetal age was calculated from 4 parameters: (a) body weight, (b) crown-rump length, (c) chest circumference, and (d) abdominal circumference. A total of 121 bovine fetuses was obtained from dams chiefly of the Hereford breed. The simple allometry equation $y = bx^k$ was found to fit the data for thyroid gland growth in relation to body weight, body length and age. The relative growth constant (k) for thyroid weight against body weight was found to be 1.0, thus indicating that thyroid weight in the fetus was nearly proportional to body weight within the limits of the empirical formula. Percentage growth rates were found to decrease with increasing age. R. P. Reece

728. The accumulation of thyroxine-like and other iodine compounds in the fetal bovine thyroid. J. WOLFF, I. L. CHAIKOFF AND C. W. NICHOLS, JR., Univ. of California Medical School, Berkeley. *Endocrinology*, 44, 6: 510-519. June, 1949.

A total of 96 thyroids was obtained from fetuses ranging in age from 53 d. to term. The

fetuses were obtained from dams chiefly of the Hereford breed. Representative portions of most of the thyroid glands were hydrolyzed on a steam bath for 12 hr. in 2N NaOH. A suitable aliquot of the hydrolysate was analyzed for thyroxine-like and non-thyroxine iodine. Measurable amounts of iodine first were detected in the fetal thyroid at 60 d. of age. The iodine content of the fetal thyroid increased steadily with increasing body weight, crown-rump length and calculated age; this increase in iodine content was greater than could be accounted for by mere increase in thyroid mass. The percentage of total iodine present as inorganic was similar to that observed in the adult thyroid gland. Thyroxine-like iodine increased steadily in the gland and the rate at which this fraction accumulated was shown to bear an exponential relation to body weight, body length and calculated age. R. P. Reece

729. Oral effectiveness of d,l-thyroxine in crystalline, monosodium and disodium forms. R. A. MONROE, AND C. W. TURNER, Univ. of Mo., Columbia. *Am. J. Physiol.* 156, 3: 381-386. Mar., 1949.

An alkaline solution of thyroxine is more readily absorbed when given orally than is thyroxine when administered in a solid form. Earlier work in comparing the oral effectiveness of various forms of thyroxine has been confusing because within given experiments the salts and crystalline form of thyroxine have not always been compared in the same physical state. The present authors eliminated these solubility differences by administering crystalline and the mono- and disodium salts of d,l-thyroxine all in the solid state. Work was done on the chick.

Using a biological assay already described (Mo. Res. Bull. 392) these workers found the mono- and disodium thyroxine possessed equal oral effectiveness and both of these forms were twice as active as the pure crystalline form. Approximately 20% of the crystalline form was absorbed and 45% of each of the sodium salts was absorbed.

V. Hurst

730. Antithyroid activity of ergothioneine. M. L. WILSON AND D. A. MCGINTY, Parke, Davis, and Co., Detroit, Mich. *Am. J. Physiol.*, 156, 3: 377-380. Mar., 1949.

Ergothioneine, the methyl betaine of mercaptomidazole, is a constituent of normal blood. There has been some evidence to show that it possesses an antithyroid activity.

The present authors compared the antithyroid activity of ergothioneine to thiouracil in the rat and monkey. Measurements in the rat included thyroid weight, thyroid I concentration, and the absorption of radioiodine by the thyroid glands.

In the monkey the absorption of radioiodine by the thyroid was studied. The ergothioneine, in large dosages, exhibited no antithyroid activity.

V. Hurst

731. Effects of alloxan administration in the calf. E. L. McCANDLESS AND J. A. DYE, Cornell Univ., Ithaca, N. Y. *Am. J. Physiol.*, **156**, 3: 355-360. Mar., 1949.

Four Guernsey bull calves, 2-3 wk. of age, were used in this experiment. Following a control period, the animals were injected intravenously with varying dosages of alloxan monohydrate (Eastman) in a 5% aqueous solution. Diabetes did not develop in these calves as measured by blood glucose. The beta cells of the islets of Langerhans, usually destroyed in other species by alloxan injections, were not injured in 2 of the 3 calves which survived the experiment. Severe renal damage was present in all animals.

V. Hurst

732. Pancreatic diabetes in the calf. E. T. COOK, J. A. DYE AND E. L. McCANDLESS, Cornell Univ., Ithaca, N. Y. *Am. J. Physiol.*, **156**, 3: 349-354. Mar., 1949.

Three male calves, 2-3 wk. of age, were used in these experiments. Preliminary determinations were made of blood and urine glucose, urinary nitrogen and urinary ketone bodies. The diet consisted of whole milk and this was supplemented with pancreatin (Merck) following pancreatectomy at 5-7 wk. of age. Pancreatectomy resulted in hyperglycemia which varied in direct proportion according to food intake. After the animals were fasted, hypoglycemia occurred more rapidly and was more extreme in depancreatized animals as compared to the controls.

Although both fed and fasted normal calves exhibited traces of glucose in the urine, the depancreatized calves showed high urine glucose levels which, however, fell sharply following fasting. Urinary nitrogen in both normal and depancreatized animals increased when they were fed, but following fasting the urinary nitrogen increased in the controls whereas it declined in the depancreatized calves. The increase in endogenous glucose from protein is slight in the depancreatized animals as compared to the controls, since the amount of nitrogen found in the urine is an index of the amount of gluconeogenesis taking place.

Gluconeogenesis can account for only a small portion of the hyperglycemia produced in the depancreatized calf, and the chief factor in producing diabetic hyperglycemia in the calf is decreased glucose utilization. Diabetes did not greatly increase the fat metabolism and glucose tolerance was lowered both by fasting and by pancreatectomy.

V. Hurst

733. Vitamins A and C concentrations in the blood plasma of ewes, their milk, and in the blood plasma of their lambs. A. L. POPE, P. H. PHILLIPS AND G. BOHSTEDT, Univ. of Wisconsin, Madison. *J. Animal Sci.*, **8**, 1: 57-66. Feb., 1949.

No significant drop in blood plasma Vitamins A and C immediately before or following parturition was noted in 18 grade ewes maintained on practical rations of alfalfa-brome grass hay and grain concentrate. Blood plasma levels of both these vitamins were highest during lactation. Blood plasma Vitamin A of lambs at birth averaged 6 γ /100 ml., increased to 20 γ with 30 hr. after birth, and ranged from 20-33 γ /100 ml. in the 13 wk. period following. Blood plasma Vitamin C was low in lambs at birth, decreased in the subsequent 4 d., then increased to a normal range.

Colostrum contained from 6 to 7 times as much Vitamin A as later milk. The Vitamin C content of colostrum did not vary from that of normal milk. No carotene was found in the plasma or colostrum of the ewes.

F. C. Fountaine

734. Transmethylation of guanidoacetic acid in beef liver autolyzates. T. L. SOURKES, Cornell Univ. *Arch. Biochem.*, **21**, 2: 265-272. Apr., 1949.

Beef liver blended in an ice water-toluene mixture and autolyzed at 20° C. for 20 hr. contains an enzyme system capable of forming creatine from guanidoacetic acid in the presence of methionine. Both ATP and oxygen are necessary for the synthesis of creatine. Boiled liver juice has an activating effect while sodium taurocholate and creatine inhibit the transmethylation of guanidoacetic acid.

H. J. Pepper

735. A polysaccharide related to the blood group substances and its reaction with borate. I. A study by electrophoresis. L. E. KREJCI, L. SWEENEY AND C. A. ZITTLE, Biochemical Research Foundation, Newark, Del. *Arch. Biochem.*, **22**, 2: 253-361. June, 1949.

A polysaccharide isolated from calf intestinal mucosa reacts with borate solutions to form diol-borate compounds of increased acidity and optical activity. The electrophoretic mobility of the polysaccharide, serologically related to the blood group A substance and known to contain L-fucose and D-galactose, was determined in both borate-free solutions and in solutions of buffer salts wholly or partially replaced by borate-boric acid mixtures. Increases in mobility paralleled a sharpening of the electrophoresis boundaries. The concentration of diol-borate is dependent upon the concentrations of both polysaccharide and borate ions. The degree of ionization of the diol-borate compounds is affected by pH.

H. J. Pepper

736. A material in bovine stomachs related to blood group B substance. S. M. BEISER AND E. A. KABAT, Columbia Univ. and Presbyterian Hospital, N. Y. J. Am. Chem. Soc., 71, 6: 2274. June, 1949.

As determined by hemagglutination-inhibition tests, substances with either blood group A, B, O, AO or BO can be obtained from different individual bovine stomachs. Analysis of purified substances reveals 5-7.2% nitrogen, 51-60% reducing sugar (as glucose after hydrolysis), 23-34% hexosamine and 1.5-5.2% methylpentose. Except for their higher content of methylpentose, hog and human substances have a similar composition. Blood group B activity was 1-5% the activity of B substance from human saliva or horse stomach. Extensive but incomplete cross reactions occurred between bovine B substances and anti-horse B, showing a higher capacity to precipitate anti-B than would be expected from observations of the hemagglutination-inhibition test. H. J. Peppler

737. The fractionation of bovine serum proteins by electrophoresis-convection. J. R. CANN, R. A. BROWN AND J. G. KIRKWOOD, Calif. Inst. of Technol., Pasadena. J. Am. Chem. Soc., 71, 5: 1609-1614. May, 1949.

The applicability of the electrophoresis-convection technique as a tool in the fractionation of naturally-occurring inhomogeneous proteins was demonstrated by the partial fractionation of bovine serum. γ -Globulins of 96% purity and β -globulins of 71% purity were separated from the fresh serum of a Hereford cow. Considerable separation of γ_1 - and γ_2 -globulin was also obtained. The electrophoresis-convection method is considered to be a fractionation tool of great importance, because large quantities of materials can be fractionated with high efficiency in a single run with relative ease of manipulation and economy of time. H. J. Peppler

Also see abs. no. 635.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

738. Report on sediment tests in milk and

cream. C. R. JOINER, Food and Drug Admin., Federal Security Agency, St. Louis, Mo. J. Assoc. Offic. Agr. Chemists, 32, 2: 324-330. 1949.

A modified method for the preparation of standard sediment discs using cow manure, garden soil and charcoal is proposed. It gave reproducible results with a given sediment mixture. Differences in appearance of pads prepared from sediment mixtures from different sections of the country were encountered. F. J. Babel

739. Report on DDT as spray residue on foods. R. H. CARTER, Bur. of Entomology and Plant Quarantine, Agr. Res. Admin., USDA, Beltsville, Md. J. Assoc. Offic. Agr. Chemists, 32, 2: 353-359. 1949.

Two methods were recommended to be adopted tentatively for determination of DDT residues in plant and animal materials: (a) determination of total organic chlorine content by the sodium and isopropanol method, (b) colorimetric determination based on the nitration of the compound and development of a blue color by sodium methylate. A procedure is given for extraction of DDT from milk samples before using the regular procedures. F. J. Babel

740. Semi-micro phenol coefficient methods for testing quaternary ammonium disinfectants. G. S. WARNER AND M. J. PELCZAR, JR., Univ. of Md., and L. S. STUART, Production and Marketing Admin., USDA, Washington, D. C. J. Assoc. Offic. Agr. Chemists, 32, 2: 401-408. 1949.

A semi-micro phenol coefficient method for determining germicidal potency of quaternary ammonium compounds is described. The method makes use of trypticase broth. Results show the minimum lethal concentration found by the semi-micro procedure was considerably lower than when the A.O.A.C. method was used. Critical quaternary ammonium germicide concentration killing times could be established more easily by the semi-micro procedure than by the A.O.A.C. technic. F. J. Babel.

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

741. *Brucella* infections. F. M. POTTINGER, JR., I. ALLISON AND W. A. ALBRECHT. Merck Report, 58, 3: 13-14. July, 1949.

Enterically coated tablets containing 2 g. Mn, 2 mg. Cu, 2 mg. Co., 60 mg. Mg and 15 mg. Zn were taken by humans at the rate of not more than 3/d. in conjunction with carefully regulated high-protein, low-sugar diets for 12 wk. or more. Many cases of relief from symptoms and improvement of blood picture are reported. Trace element administration to a herd of cattle is reported to have increased the number of viable calves, increased milk production and improved the breeding record. Data are not presented.

F. E. Nelson

742. The milk ring test for detecting *Brucella* agglutinins in cow's milk. I. F. HUDDLESON AND C. CARRILLO, Michigan State College, East Lansing. Vet. Med., 44, 6: 240-243. June, 1949.

A complete description is given for the preparation of *Brucella* antigen for the detection of *Brucella* agglutinins in milk by the Fleishhauer ring test. A transparent plastic block bored with 0.25 in. holes serves as test tubes and rack. Soda straws were used instead of glass pipettes. The milk samples mixed with antigen are incubated at room temperature and the test read 1 to 6 hr. later. The ring test may be a valuable aid in conjunction with the blood agglutination test for detecting brucellosis.

B. B. Morgan

743. Procaine penicillin G levels in udder during treatment of chronic mastitis. W. D. BOLTON, J. M. FRAYER, J. H. CADY AND E. F. WALLER, Vermont Agr. Expt. Station, Burlington. Vet. Med., 44, 6: 244-245. June, 1949.

Twenty-eight cows infected with mastitis were divided into 3 groups and treated with procaine penicillin G bougies. Of these groups, 18 received 25,000 units/quarter, 6 received 50,000 units and 6 received 75,000 units. A total of

112 quarters were treated. Milk samples for bacterial colony counts and penicillin assays were collected before treatment and 1, 4, 6 and 8 hr. after treatment. The number of colonies were reduced from a maximum of 36,000 to 10 and the maximum number of units of penicillin/ml. dropped from 225 to 10/quarter. The amount of penicillin remaining at the end of 8 hr. was in excess of that required to inhibit streptococci.

B. B. Morgan

744. Aureomycin in the treatment of staphylococcal mastitis in cows. E. C. McCULLOUGH, J. S. KISER AND H. MIGAKI, Washington State College, Pullman. Vet. Med., 44, 6: 253-258. June, 1949.

A group of 16 cows representing 61 staphylococcal mastitis infected quarters was treated. Three cows received 100 mg. of aureomycin in 20 ml. sterile saline/quarter; 3 others received 100 mg. in 7.5 g. of an ointment base (10% lanolin, 22.5% mineral oil and 67.5% white petrolatum). Additional treatments were given to several flare-up cases. Twenty-four quarters were treated with 12 quarters (50%) apparently cured. A second group of 6 cows was treated with 150 mg. of aureomycin in 30 ml. sterile saline/quarter by intramammary infusion. The treatment was repeated after 48 hr. Four cows received sulfamethazine orally. Of 23 quarters involved, 60.8% were cured. The last group of 4 cows was given 200 mg. of aureomycin in 15 g. of ointment; treatment was repeated at 72 hr. Of 14 quarters treated, 12 (85.7%) apparently were cured. Assays of aureomycin in the milk revealed between 12 to 50 γ /ml. at 24 hr., 6 to 25 at 48 hr. and 1 to 2 γ at 72 hr. No evidence of irritation or reduction in milk flow due to the drug was observed.

B. B. Morgan

745. Preliminary report on use of sulfamethazine and penicillin in bovine mastitis. R. W. FULLER, State Institution Farms Bureau, Batavia, N. Y. Vet. Med., 44, 3: 103-107. Mar., 1949.

Infected quarters of approximately 127 cows were determined by physical examination, strip

cup and bromthymol blue tests. The routine treatment was the infusion into each infected quarter of 50 ml. of a 10 to 25% sterile solution of sodium sulfamethazine by weight/volume with 25,000 or 50,000 units of penicillin. Two infusions at 24 hr. intervals or 4 infusions at 12 hr. intervals were given. A 94.6% clinical recovery of mastitis by the sulfamethazine-penicillin treatment was obtained. Milk was available for human consumption 3 to 5 d. after treatment. Toxic symptoms or udder irritation were not observed.

B. B. Morgan

746. Mastitis in dairy cattle. J. W. CUNKELMAN, Fort Dodge, Iowa. *Vet. Med.*, **44**, 5: 207-209. May, 1949.

A brief review of mastitis pointing out that during the past 1.5 yr. most of the research has been toward treatment of the disease. Various control measures are discussed. B. B. Morgan

747. Veterinary practitioners and community health. J. H. STEELE, U. S. Public Health Service, Atlanta, Ga. *Vet. Med.*, **44**, 5: 192-195. May, 1949.

A brief review of the health policies involving man and animals in the United States is presented. Emphasis is placed on the role of the veterinarian in regard to milk sanitation and food inspection. The U.S.P.H.S. has established veterinary research units in research and communicable disease control. The main objectives of this program are the pasteurization of dairy products from disease free animals and veterinary inspection of all meat and dairy products.

B. B. Morgan

748. Diagnosis and control of mange in dairy cattle. H. H. SCHWARDT, Cornell Univ., Ithaca, N. Y. *J. Econ. Entomol.*, **42**, 3: 444-446. June, 1949.

Dairy cattle mange diagnosis during early stages is important. Then prompt treatment can prevent serious damage. Cattle died from mange; others lost condition and fell far below normal production. In the north most serious cases occur during cold weather.

Mange may be confused with several other skin disorders. Definite diagnosis may be made by microscopic examination of deep skin scrapings at the edges rather than the center of active lesions. Scrapings must be deep enough to draw blood if sub-dermal Sarcopitic mites are to be found. Technic for concentrating a possibly few mites is described. There is discussion of Sarcopitic, Chorioptic, Psoroptic and Demodectic mites. High pressure (400 lb.) spraying equipment with at least 2 gal./animal, for coverage of all

external body parts, is described. Four applications at weekly intervals with either (a) lime sulphur solution at 1:15 dilution, or (b) 20 lb. wettable sulphur in 100 gal. water gave mange-free herds 8 mo. after treatment. Benzene hexachloride was used, but no conclusive control evidence presented.

Milk from cattle sprayed with 6 lb. of 6% gamma benzene hexachloride/100 gal. water contained about 4 p.p.m. of benzene hexachloride a few hr. after treatment. No residue was detected after about 1 wk. E. H. Fisher

749. Poisoning of farm animals by the marsh ragwort. W. C. EVANS AND E. T. R. EVANS. *Nature*, **164**, 4157: 30. 1949.

The death of cows, horses, etc. on pastures or cured hay containing marsh ragwort (*Senecio aquaticus*) is caused by an alkaloid named aequicene by the authors. R. Whitaker

BUTTER

O. F. HUNZIKER, SECTION EDITOR

750. Observations on butteroil. C. W. DECKER, State College of Washington. *Natl. Butter Cheese J.*, **40**, 8: 32-34. Aug., 1949.

A description of the American method of processing butteroil from cream and the Australian and New Zealand method of processing it from butter is given. A high quality cream is essential to avoid off-flavors in the finished product. A good quality butteroil can be made from butter of low quality caused by physical defects, high free fatty acids, neutralization or protein decomposition. Butteroil of satisfactory quality cannot be made from butter that is defective due to either metallic flavors, fishiness, tallowiness or excessive oiliness. Butteroil can be kept for a period up to a year at ordinary warehouse temperatures or for a longer period if held at 0° F. This product may be used for almost any food product where cream or butter is used. Butteroil may be used in the manufacture of preserved butters. Another possible use is for overseas shipments as a source of butterfat in reconstituted milks.

H. E. Calbert

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

751. Production of devitaminized casein by solvent extraction. S. M. WEISBERG AND J. GREENSPAN (assignors to National Dairy Research Labs., Inc.). U. S. Patent 2,477,505. 6 claims. July 26, 1949. *Official Gaz. U. S. Pat. Office*, **624**, 4: 1211. 1949.

Casein is devitaminized by maintaining the pH in the range 4.7 to 6.0 and extracting the vitamins with methanol. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

752. Thermometer with mounting well. R. J. WINNING (assignor to Sheffield Farms Co.). U. S. Patent 2,475,211. 3 claims. July 5, 1949. Official Gaz. U. S. Pat. Office, 624, 1: 232. 1949.

To overcome the delay in reaching the correct temperature of a thermometer inserted in a fixed well in the wall of pasteurizing and other tanks, vats, etc., 2 holes are provided in the well on the outside of the tank. One hole, located on the bottom side of the well is for draining while the other hole, located on top, is for introducing a liquid into the well to provide better contact between the thermometer and the well.

R. Whitaker

753. Meeker tube holder. H. J. EASTON. U. S. Patent 2,477,366. 2 claims. July 26, 1949. Official Gaz. U. S. Pat. Office, 624, 4: 1175. 1949.

An adjustable spring arm is attached to the handle of the milk receiver of a milking machine and holds the rubber tubes from dragging in the litter on the floor of the stall. R. Whitaker

754. Hoist. S. H. HALL (assignor to DeLaval Separator Co.). U. S. Patent 2,476,192. 5 claims. July 12, 1949. Official Gaz. U. S. Patent Office, 624, 2: 615. 1949.

This hoist is designed for lifting milk cans in dairy barns equipped with a vacuum line. The vacuum is employed to operate a piston in a cylinder, which, through a system of pulleys, raises and lowers the milk can into cooling tanks, on trucks, etc. The whole mechanism is mounted on a vertical cylindrical support fastened to floor and ceiling. R. Whitaker

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

755. The influence of corn starch upon roughage digestion in cattle. W. BURROUGHS, P. GERLAUGH, B. H. EDGINGTON AND R. M. BETHKE, Ohio Agr. Expt. Station, Wooster. J. Animal Sci., 8, 2: 271-278. May, 1949.

The effects of adding varying amounts of mineralized starch to basal rations of either corn-cobs, alfalfa hay, or corn-cobs and alfalfa hay on the digestibility of the dry matter in the roughage were determined in 5 series of digestion trials.

Apparent digestion of the dry matter of basal rations of corn-cobs, or of corn-cobs and alfalfa hay was decreased with each increment of starch. The addition of starch had no significant effect on the digestibility of the dry matter of alfalfa hay basal rations in one series. In 2 of the alfalfa hay series only slight decreases in dry matter digestibility were noted when starch was added to the ration. F. C. Fountaine

756. The nutritive value of green berseem (Egyptian clover), hay and silage. A. GHONEIM, M. T. EL-KATIB AND A. A. BADR. Fouad I Univ., Giza, Egypt. J. Animal Sci., 8, 2: 279-285. May, 1949.

Chemical analyses and digestion trials with sheep were used to determine the nutritive value of berseem (*Trifolium alexandrinum*) fed as fresh forage, hay and as silages made as follows: (a) untreated-unwilted, (b) 1% molasses, (c) wilted, and (d) A. I. V. The starch equivalent and digestible protein were higher in green berseem than in hay or any silage. A. I. V. silage was superior to hay in starch equivalent and equal to hay in digestible protein. Hay was superior to silages made by other methods, in both starch equivalent and digestible protein.

F. C. FOUNTAINE

757. The effect of crude soybean lecithin on the absorption and utilization of Vitamin A fed prepartum to the ewe and sow. H. D. EATON, J. A. CHRISTIAN, F. C. DAUGHERTY, A. A. SPIELMAN AND I. D. MATTFERSON, Univ. of Connecticut, Storrs. J. Animal Sci., 8, 2: 224-233. May, 1949.

Supplementation of a normal prepartal ration for sows and ewes with Vitamin A with or without soybean lecithin increased the Vitamin A content of the blood of the dams, and in the blood and livers of their offspring at birth and at 30 d. of age. The addition of lecithin to the Vitamin A supplement did not materially increase the content of Vitamin A in the blood or livers over that of animals receiving Vitamin A concentrate alone. F. C. Fountaine

Also see abs. no. 749.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

758. A study of the metabolic activity of bull semen and spermatozoa in relation to their fertilizing ability. D. GHOSH, L. E. CASIDA AND H. A. LARDY, Univ. of Wisconsin, Madison. J. Animal Sci., 8, 2: 265-270. May, 1949.

The respiration rate (cmm. of O_2 uptake/ 10^6 cells/hr.) of 4 mature Guernsey and 4 mature Holstein bulls ranged from 3 to 12.55, a lower rate than obtained in previous studies. There was no correlation between respiratory metabolism of spermatozoa and their fertility.

F. C. Fountaine

759. An hereditary digital anomaly of cattle. S. W. MEAD, P. W. GREGORY AND W. M. REGAN, Univ. of California, Davis. *J. Heredity*, 40, 6: 151-155. June, 1949.

Hoofs that were small and slightly malformed were associated with probable modifications in the metacarpals, carpus and tarsus in 5 Jerseys of both sexes. Pain was manifested when affected animals were on their feet, with more discomfort in the fore than in the hind feet. The condition was observed at ages of 2 to 4 mo. and became progressively worse. Feeding, health and management all were excluded as possible causative factors. All affected animals descended in one or more lines from each of 2 bulls that had a common great-grandparent. A single autosomal recessive gene is indicated as the genetic conditioner for this anomaly. L. O. Gilmore

760. Dwarf cattle for the tropics. C. G. ARRILAGA, Univ. of Puerto Rico. *J. Heredity*, 40, 6: 167-168. June, 1949.

Proportionate dwarfism has been observed in a few cases in the cattle of Puerto Rico. Inherited dwarfism (recessive) is distinguished from the condition produced by environmental causes. The possible significance of genetic dwarfs possessing high performance ability for use in the mountainous regions of tropical America is indicated. L. O. Gilmore

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

761. Milking parlor. H. B. BABSON AND C. A. THOMAS (assignors to Babson Bros. Co.). U. S. Patent 2,477,035. 7 claims. July 26, 1949. Official Gaz. U. S. Pat. Office, 624, 4: 1091. 1949.

This milking parlor is characterized by having the cows on a level above the operator's floor for convenience in milking. R. Whitaker

762. Stock watering fountain. A. F. KLINZING. U. S. Patent 2,476,876. 1 claim. July 19, 1949. Official Gaz. U. S. Pat. Office, 624, 3: 906. 1949.

A bowl shaped fountain for watering farm animals, such as cows. A hinged member, depressed by the animal's nose, actuates a valve and admits water to the bowl. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

763. Carton. B. A. RAFOTH (assignor to Marathon Corp.). U. S. Patent 2,475,294. 1 claim. July 5, 1949. Official Gaz. U. S. Pat. Office, 624, 1: 254. 1949.

A paper carton for ice cream and other food products similar in shape to the regular pail type container with a spoon or dispensing implement held to the top cover by an extension panel, so cut and folded to both hold the spoon and lock the cover of the carton. R. Whitaker

MILK SECRETION

V. R. SMITH, SECTION EDITOR

764. The effect of the plane of nutrition on the composition of sow's colostrum and milk. J. P. BOWLAND, R. R. GRUMMER, P. H. PHILLIPS AND G. BOHSTEDT. Univ. of Wisconsin, Madison. *J. Animal Sci.*, 8, 2: 199-206. May, 1949.

Colostrum from sows fed on pasture during gestation was higher in fat, and lower in S.N.F., protein and lactose than that of sows carried on dry lot during gestation. There was no difference between groups in the T.S. and ash of the colostrum. Milk sampled weekly from the 1st to the 8th wk. from sows maintained on pasture was lower in T.S. but was not appreciably different in S.N.F., protein, ash and lactose than milk from sows fed on dry lot. No breed differences were noted. F. C. Fountaine

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

765. Tocopherol (Vitamin E) deficiency among sheep fed natural feeds. F. WHITING, J. P. WILLMAN AND J. K. LOOSLI, Cornell Univ., Ithaca, N. Y. *J. Animal Sci.*, 8, 2: 234-242. May, 1949.

Colostrum and milk of ewes fed a prepartal ration of alfalfa hay and cull beans, even when supplemented with tocopherols, contained insufficient tocopherol to prevent stiff lamb disease. Administration of tocopherols to the lambs cured the condition. The incidence of muscle dystrophy was higher in lambs of ewes fed a prepartal ration including alfalfa hay than in those of ewes fed mixed or grass hay.

F. C. Fountaine

766. Supplemented milk diets for young pigs in cages. J. A. WEYBREW, H. A. STEWART, G. MATRONE AND W. J. PETERSON. North Carolina

Agr. Expt. Station, Raleigh. J. Animal Sci., 8, 2: 207-223. May, 1949.

Three whole milk diets supplemented with minerals and cod liver oil were compared for pigs from 2 d. to 8 wk. of age. When measured by growth, reconstituted whole milk solids was equal to reconstituted skim milk solids plus butter, and both were superior to evaporated milk. There was no difference in efficiency of the 3 diets. Pigs on each of the 3 diets made better gains than those nursing well fed sows.

F. C. Fountaine

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

767. Residual action of organic insecticides against stable flies. G. W. EDDY AND W. S. MCGREGOR, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 3: 547-548. June, 1949.

Laboratory tests with 11 recently developed organic insecticides were made on stable flies to determine speed of knock-down and kill, and length of effectiveness. Screen cages were dipped in 1% solution of toxicant in acetone. One cage was held indoors while the other was exposed to the weather. Tests were made 1, 7, 14, 50 and 126 d. after treatment. Flies were held in cages up to 48 hr.,

DDT and methoxychlor were fastest acting, and toxaphene and chlordan were slowest, in 24 hr. check. Methoxychlor and the bromine analog of DDT were superior to others in knock-down and duration of effectiveness. Parathion, compound 118 and toxaphene retained toxicity longer than dichlorodiphenyl dichloroethane, gamma benzene hexachloride (95%), compound 153, heptachlor or chlordan. E. H. Fisher

768. Toxicity to house flies of synthetic compounds of the pyrethrin type in relation to chemical structure. W. A. GERSDORFF, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 3: 532-536. June, 1949.

Laboratory tests with house flies showed some new synthetic compounds related to pyrethrins to be more toxic than a mixture of pyrethrins contained in the ordinary pyrethrum-kerosene extract. E. H. Fisher

769. Fumigating properties of several new insecticides. R. A. HOFFMAN AND A. W. LINDQUIST, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 3: 436-438. June, 1949.

Laboratory tests with house flies showed several residual, contact insecticides to have a fumigating effect. A 100 mg./ft.² treatment on the inside of wide-mouth qt. jars was made. Flies were held in similar untreated jars immediately above these for 6 hr. Benzene hexachloride, parathion, chlordan and fluorine analog of DDT caused complete kill of flies within 24 hr. after the 6 hr. exposure to their vapors. Toxaphene and TDE vapors gave high mortalities, but DDT killed none. A 10 mg./ft.² treatment was also tested.

E. H. Fisher

770. The residual property of DDT as influenced by temperature and moisture. A. F. BURGESS AND H. L. SWEETMAN, Univ. of Massachusetts. J. Econ. Entomol., 42, 3: 420-423. June, 1949.

House fly mortality was used to measure the effects of moisture and temperature on DDT, applied in kerosene solution to screens. Treated screens held at 37° C. and 60 to 75% relative humidity decreased in toxicity more rapidly than those held at 23° C. and 25 to 40% humidity.

E. H. Fisher

771. Failure of DDT to control houseflies. W. V. KING AND J. B. GAHAN, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 3: 405-409. June, 1949.

House flies from several natural sources, including dairy barns, were compared with laboratory-reared ones for resistance to some insecticides. Laboratory tests with only 25 mg. DDT/ft.² of treated panel showed 70% mortality in 8 to 67 min. for flies from natural sources, and 1.1 to 3.19 min. for laboratory flies.

Chemical analysis of several DDT preparations revealed no inferior quality. Biological tests were not reported.

With cage tests on dairy barn walls, variation in mortality showed uneven DDT application.

Deposits of DDT, technical benzene hexachloride, and partially refined benzene hexachloride showed fly repellency when used at rates much greater than usually recommended, chlordan and methoxychlor did not. Tests indicated there was greater fly resistance to DDT than to methoxychlor, chlordan or benzene hexachloride.

E. H. Fisher

772. Insecticides and the food law. C. W. CRAWFORD, Deputy Commissioner of Food and Drugs. J. Econ. Entomol., 42, 3: 564-566. June, 1949.

The Federal Food, Drug and Cosmetic Act of 1938 prohibits interstate traffic in adulterated foods. Some adulteration tolerances in or on foods are in effect, and others may be set, if it

can be shown that "—such substance is required in the production thereof or cannot be avoided by good manufacturing practice." Interpretation of the Act indicates there should be insurance that the total of toxic substances in all items constituting our diets will be held at safe levels. This is in contrast to tolerance considerations regarding safety of a single adulterated food.

Since the advent of new insecticides, beginning with DDT, information on public health problems related to these materials has not kept pace

with the development and use of them. In addition to proving chemicals which may be used in connection with food production to have excellent insecticidal value, it is also necessary to devise quantitative analytical methods, determine toxicity to man and other animals and determine the quantities to which man and other animals may be exposed. Among other answers needed are whether the insecticide is translocated in plants, excreted in milk of animals which consume it, or cumulative in animal tissue. E. H. Fisher

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEWS

773. Monographs on the progress of research in Holland during the war. Chemical and physical investigations on dairy products. H. EILERS, R. N. J. SAAL and M. VAN DER WAARDEN. Elsevier Publishing Co., Inc., New York, N. Y. 215 pp. \$4.00. 1947.

This monograph, the 12th of the series which Elsevier is publishing on various aspects of Dutch research during the war, contains 3 treatises summarizing the results of investigations on the chemistry of dairy products which were carried out by the Amsterdam Laboratory of the N. V. de Bataafsche Petroleum Maatschappij at the instigation of the General Netherlands Dairy Union. These results previously had been published in somewhat greater detail in Dutch by the authors and their associates in *Verslagen Landbouwkundige Onderzoekingen*, vol. 50 and 52. The three topics discussed are: (1) the colloid chemistry of skim milk (Eilers), (2) the oxidation-reduction potential of milk and of butter plasma (Saal) and (3) the chemical processes underlying the deterioration of butter in cold storage (van der Waarden).

Eilers discusses the composition, state, properties and behavior of the colloidal micelles (Ca caseinate-phosphate and serum protein) of skim milk. The discussion of caseinate is by far the better; the section on milk serum proteins shows evidence of lack of access to foreign literature after 1940. Experimental data are presented on the viscosity of skim milk and the effect of heat treatment thereon, which is shown to involve the caseinate micelles and the serum proteins. The behavior of milk upon concentration, both with and without the addition of sugar, is discussed in detail, particularly with regard to viscosity and distribution of phosphate among the phases.

Saal's chapter on oxidation-reduction deals with the factors determining the potential and with the relation of changes in the potential to development of oxidized flavors in milk. The potential of fresh raw milk (average +0.27 v.) is determined principally by the oxygen and ascorbic acid

contents. It is reduced by heat treatment and increased by addition of Cu or Fe salts which also promote oxidized flavor development. The potential of butter plasma is high but its magnitude is not related to flavor of the butter.

Van der Waarden presents evidence that fishy flavors in storage butter arise from oxidative processes, rather than from hydrolysis of lecithin to trimethylamine. Attempts to isolate the off-flavor compound(s) were unsuccessful, although such compound(s) were prepared in a highly concentrated form.

This monograph contains much rather detailed information that should be of considerable interest to workers in the fields covered. It is well-organized and fairly well-written. The translation is not always as smooth as possible and there are a number of errors, particularly in spelling, but these defects do not detract greatly from the value of the book. The paper binding is entirely inadequate. R. Jenness

774. Practical Dairy Bacteriology. PAUL R. ELLIKER. McGraw-Hill Book Co., Inc., New York, N. Y. 391 pp. \$4.00. 1949.

This excellent elementary text and reference book presupposes no previous knowledge of either bacteriology or chemistry. The earlier portions of the book are concerned with the simple fundamentals of bacteriology, and subsequent chapters relate these fundamentals to the handling of the various dairy products. Considerable information on the technology of the different dairy products is presented in order that the science may be related intimately to the practice. References to more detailed treatments of the various topics are given at the end of each chapter and add considerably to the usefulness of the book.

The style of writing used presents the material in very understandable form with a minimum of highly technical vocabulary. The illustrations are unusually satisfactory and the captions are much more explanatory than one encounters commonly; however, lack of any indication of magnification factor or other basis of size in or

with the photomicrographs of microorganisms is unfortunate in the opinion of the reviewer. The chapters on "Methods used to determine sanitary quality of milk and milk products" and "Cleaning dairy equipment" probably will be found particularly useful by many, as these presentations fill a definite need in a very satisfactory way.

F. E. Nelson

775. Annual review of biochemistry, vol. XVIII. J. M. LUCK, ed. Annual Reviews, Inc., Stanford, Calif. 739 pp. 1949.

The chapters in this edition are: *Biological Oxidations*, by P. W. Preisler and F. E. Hunter, Jr.; *Proteolytic Enzymes*, by E. L. Smith; *Non-oxidative, Nonproteolytic Enzymes*, by K. Myrbäck; *Carbohydrate Chemistry*, by D. J. Bell; *Chemistry of the Lipids*, by J. A. Lovern; *Chemistry of Amino Acids and Proteins*, by H. P. Lundgren and W. H. Ward; *Nucleoproteins, Nucleic Acids, and Derived Substances*, by J. N. Davidson; *Metabolism of the Lipids*, by A. L. Lehninger; *Chemistry of Neoplastic Tissue*, by E. Boyland; *Metabolism of Proteins and Amino Acids*, by A. Neuberger; *Intermediary Metabolism of Phosphorous Compounds*, by F. Lipmann and N. O. Kaplan; *Carbohydrate Metabolism*, by H. G. Wood and V. Lorber; *Mineral Metabolism (Fluorine and Other Trace Elements)*, by J. F. McClure; *Chemistry of the Hormones*, by A. Wettstein and F. Benz; *Fat Soluble Vitamins*, by P. L. Harris; *Water Soluble Vitamins*, by E. L. R. Stokstad and T. H. Jukes; *Nutrition*, by A. Keys; *Metabolic Inhibitors*, by R. J. Winzler; *Chemistry of Antibiotics*, by O. Wintersteiner and J. D. Dutcher; *Insect Biochemistry*, by V. B. Wigglesworth; *Nitrogenous Constituents of Plants*, by A. G. McCalla; *Organic Acids of Plants*, by T. A. Bennet-Clark; and *Mineral Nutrition of Plants*, by C. H. Wadleigh.

These reviews cover material appearing during late 1947 and nearly all of 1948, in many instances. However, where the subject has not been reviewed for some time or where the reviewer has employed an approach different from that used previously, the period covered may be considerably longer and extend back as far as 1940 in several cases. The quality of the reviews is what one has come to expect of this publication, and the same is true of the indices, format, etc. This is a very useful reference volume.

F. E. Nelson

776. Manuale Lactis. M. E. SCHULZ and E. VOGEL, ed. Verlag Hans Carl, Nürnberg, Germany (U. S. Zone). 1948.

Nine sections of this publication have been received. Each section contains abstracts of original

articles in that specific field, appropriate subsections being used for further classification. The sections available are: *Milch in der Landwirtschaft (Milk in Agriculture)*, *Milch als Nahrungsmittel (Milk as Food)*, *Milch und Milchprodukte (Milk and Milk Products)*, *Milch in der Molkerei (Milk in the Dairy)*, *Butter und Fette (Butter and Fat)*, *Käse (Cheese)*, *Nebenprodukte (By-products)*, *Bau- und Maschinenwesen (Construction and Machinery)* and *Molkerei-Hilfstoffe (Dairy Supplies)*. Many of the abstracts are original, while others are copied from other abstracting sources. All abstracts are in German. The literature of the world seems to be covered quite well from the manufacturing side. Some of the articles abstracted date back to 1939 and quite a few war-time articles are included. One observes a number of errors which it is hoped can be reduced in future issues. Appearance of an index will help to make the material more useable. Prices available for the individual sections range from 8.90 to 20.10 German marks. The nine sections available occupy 1085 pages.

F. E. Nelson

777. The better utilization of milk. R. C. HUTCHINSON Angus & Robertson, Ltd., Sydney, Australia. 218 pp. 25/-. 1948.

Milk and the various products which are manufactured from it are discussed with particular emphasis upon the influence of the composition of the various products and of the processing operations upon food value. The treatment necessarily is brief because of space limitations, but numerous references to the literature provide ample opportunity for finding more information when the reader so desires. The indexing is very extensive. Although the preponderance of the material covered is Australian or from the British Empire, much data from the United States is included. The book is written well and well printed.

F. E. Nelson

778. Sugar: its production, technology and uses. ANDREW VAN HOON. Ronald Press Co., New York, N. Y. ix + 155 pp. \$3.00. 1949.

The author has presented very well a resumé of the sugar industry in "readable and understandable form the essential facts about the growing, processing and refining of sugar, the scientific basis of its technology, and its many and diverse uses. . . ." In 6 chapters, the subjects of Sugar (its chemical and physical characteristics), Production of Cane and Beet Sugars, Commercial and Trade Aspects, By-Products and History are covered. Over a third of the book is devoted to an interesting description of sugar manufacturing methods.

The book will help those who use sugar in dairy and food products to have an appreciation of its nature and origin. It is written for those with technical knowledge to better appreciate sugar, rather than as a reference on technical properties of sugars, such as they are used in foodstuffs.

K. G. Weckel

779. Experimental Immunochemistry. E. A. KABAT and M. M. MAYER. Charles C. Thomas, Springfield, Ill. 567 pp. \$8.75. 1948.

The emphasis is upon laboratory technics for use in the indicated field branching out into related areas with which the field of immunochemistry has common boundaries and in which many of the applicable technics have been developed. The emphasis is upon the quantitative approach, wherever this is applicable. The material is divided into 5 parts: Immunological and immunochemical methodology, Applications and uses of quantitative immunochemical methods, Chemical and physical methods and special procedures used in immunochemistry, Preparations and Appendix. A good index is provided and there are many references to original publications at appropriate places. This volume should prove an excellent reference for all working in the immediate field and to many working in other areas of biological chemistry.

F. E. Nelson

780. C. O. Jensen. Selected papers. Vol. I, 1886-1908. M. CHRISTIANSEN and H. O. SCHMIT-JENSON, ed. Einar Munksgaard, Copenhagen, Denmark. 681 pp. (Price not given). 1948.

In addition to a bibliography, 37 original papers (8 published in collaboration with others) are included. Thirteen papers previously not translated to a major language have been translated to English for this publication and 23 originally were published after translation to German, in which language they appear in this publication. The papers are concerned largely with animal diseases, although there are 2 on milk bacteriology and several on special groups of bacteria. Many of these papers would not be available to the majority of the interested people except for this republication. Although the illustrations have suffered somewhat because they had to be reproduced from reproductions, their quality is surprisingly good.

F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

781. Studies on repeated vaccination of cattle with *Brucella abortus* strain 19. I. The agglu-

tinin response of animals vaccinated as calves and revaccinated as young adults. D. T. BERMAN and B. A. BEACH, Wis. Agr. Expt. Sta., Madison. *Am. J. Vet. Research*, 10, 36: 208-213. July, 1949.

Three groups of 20 to 22 heifers were vaccinated at 8 mo., 1 group revaccinated at 14 mo. and another group revaccinated at 20 mo. The heifers were managed as a single group. All heifers were bred at 15 to 18 mo. Detailed information as to blood titer and reproductive function was kept. Following revaccination at both 14 and 20 mo., the blood titer rose rapidly to over 1:1,000 and then gradually declined, leveling off at a considerably higher average than found in the singly vaccinated group. All except 1 in each revaccinated group was below 1:100 at the termination of the first pregnancy. Revaccination did not appear to have any deleterious effect on the pregnancy. A virulent *Brucella* infection, not Strain 19, was detected in animals in each group, resulting in some abortions during the observation period. E. W. Swanson

782. Relation of human and bovine brucellosis in Minnesota. D. S. FLEMING, Minn. Dept. of Health, Minneapolis, and M. H. ROEPKE, Univ. Farm, St. Paul. *Pub. Health Repts.*, 64, 33: 1044-1051. Aug. 19, 1949.

From 1937 to 1947 the number of reported cases of human brucellosis increased 4- to 10-fold in those districts in Minn. made up of counties not under the area plan of bovine brucellosis control. During this same period there was no appreciable increase in the number of reported cases in the district comprising all 21 counties under an area plan. This difference could not be attributed to fluctuations in cattle population since the relative increase in number of cattle during the war was much the same in all districts. The authors believe that the area plan of bovine brucellosis control is of considerable benefit from the public health standpoint.

D. D. Deane

783. An epidemiologic study of brucellosis in Minnesota. R. L. MAGOFFIN, National Institute of Health; P. KOBLER, Minn. Dept. of Health, Minneapolis; W. W. SPINK, Univ. of Minn., Minneapolis, and D. FLEMING, Minn. Dept. of Health, Minneapolis. *Pub. Health Repts.*, 64, 33: 1021-1043. Aug. 19, 1949.

The number of cases of human and bovine brucellosis in Minn. has increased rapidly since the period before World War II. *Br. abortus* was the causative organism in 85% of the 268 human cases studied from Jan., 1945 through June, 1948. The remaining 15% were divided almost

equally between *Br. suis* and *Br. melitensis* and occurred mainly among meat-packing plant employees handling infected swine. Nearly half the cases were found in individuals whose work involved handling livestock or slaughtered animals while about three-fourths had some contact with farm animals. The only source of the infection in about one-fourth of the cases was raw milk. These cases, equally divided between males and females, were more evenly distributed over all ages than those cases caused by animal contact. The agglutinin test was found to be a reliable diagnostic aid in active brucellosis. The authors believe that prevention of the human disease depends upon eradication of the disease in animals.

D. D. Deane

784. Bovine mastitis associated with beta-haemolytic group C streptococci. J. C. Buxton, Vet. Investigation Centre, Sutton Bonington, Leics. *British Vet. J.*, 105, 4: 107-114. April, 1949.

A brief report is given on 2 outbreaks of bovine mastitis in widely separated herds in which hemolytic group C streptococci were isolated from the milk. In 1 herd of 46 cows treated with penicillin the disease continued to spread. Bacteriological examinations revealed that 41 animals were harboring hemolytic streptococci, 10 of which were group C. The remaining animals were infected with *S. agalactiae*. Each infected animal received 50,000 units of penicillin in 50 cc. of boiled water in all quarters, once daily for 3 d. After 3 d. animals showing streptococci were treated again. *S. agalactiae* was reduced 90%, while only 11% reduction occurred in the animals infected with the group C streptococci. Two further courses of treatment reduced the incidence.

In the second herd of 35 cows, 25 were infected with streptococci. 9 cows with 18 involved quarters showed *S. agalactiae*, while 16 cows with 41 infected quarters showed group C streptococci. The treatment was increased to 100,000 units for 3 d. The quarters infected with *S. agalactiae* were reduced 66%, while 58% of the C group streptococci were clinically cured. It was pointed out that under field conditions *S. pyogenes* mastitis would be difficult to diagnose and heavy infections would fail to respond to ordinary penicillin treatment.

B. B. Morgan

785. Penicillin ointment in the treatment of chronic bovine mastitis. A. S. SCHLINGMAN and MARY C. MANNING, Parke, Davis and Co., Detroit, Mich. *Vet. Med.*, 44, 9: 382-388. Sept., 1949.

Penicillin (calcium salt) was incorporated at the rate of 50,000 units in 3.6 g. of a bland

ointment base in collapsible tubes and used for treatment of a herd of purebred Holstein-Friesian cattle infected with chronic bovine mastitis. Eleven cows involving 25 quarters were treated. Infected quarters were determined by physical examination, strip cup, bacteriological examinations employing bacto-tryptose agar slants, 5% rabbit blood agar plates, Hotis tests and microscopic examinations. Treatment was given at various intervals into all 4 quarters/cow regardless of the number of infected quarters. As a rule, 50,000 units of penicillin ointment was used per quarter, but on several occasions 100,000 units were employed. A range of 1 to 10 treatments was necessary to free 22 of 25 streptococcus- or staphylococcus-infected quarters. Bacteriological examinations were made up to 12 wk. after the last treatment. Three cases of *coli-aerogenes* mastitis were treated with streptomycin with apparently good results. Penicillin in an ointment base was no more irritating to the udder than normal saline or distilled water. Complete protocols are given on all cows treated.

B. B. Morgan

786. The sensitiveness of pathogenic staphylococci isolated from animals to penicillin. H. FARRAG, Cairo Veterinary College, Egypt. *British Vet. J.*, 105, 2: 64-66. Feb., 1949.

The sensitivity to penicillin of 25 cultures of staphylococci isolated from mastitis cows, septic wounds in horses and otitis media in dogs was tested *in vitro*. The test involved the use of a standard inoculum of a 24-hr. broth culture on agar plates containing various units of penicillin. All of the cultures were *Staphylococcus aureus* except 3 which were *S. albus*. All of the strains were found to be penicillin sensitive. In 1 experiment the resistance of staphylococci could be increased artificially up to 220 times, consequently, naturally occurring resistance to penicillin may be encountered.

B. B. Morgan

787. Human tuberculosis of bovine origin. Its prevention is still an urgent problem. S. G. TIPPETT, Nordrach-upon-Mendip Sanatorium, Bristol, England. *British Vet. J. (formerly Vet. J.)*, 105, 1: 14-18. Jan., 1949.

A brief review of human tuberculosis of bovine origin in England from the medical point of view is given. Only 16% of the cattle in Eng. are certified free from tuberculosis. A recent survey indicated that 1,500 to 2,000 persons die of tuberculosis of bovine origin annually and approximately 4,000 new cases occur every year. Methods used in the U. S. for eradication of bovine tuberculosis and pasteurization of milk were cited as definite proof that the disease can be prevented.

The incidence of tuberculosis in cattle in Eng. may run as high as 35%. A strong appeal was made for community action of livestock owners to demand bovine tuberculosis eradication.

B. B. Morgan

788. The occurrence of *Hypoderma* larvae in the spinal canal of cattle. W. O. HABERMAN, B. B. MORGAN and R. J. DICKE. J. Agr. Research, 78, 12: 637-640. June 15, 1949.

Of 982 larvae removed from 293 infected spinal canals, 975 were identified as *Hypoderma bovis* and 7 as *H. lineatum*. The maximum number of larvae removed from a single infected spinal canal was 21. The length of the larvae ranged from 5 to 17 mm. and a progressive increase in the average length was noted from Sept. (7.5 mm.) to May (15.6 mm.). In Wisconsin, *H. bovis* was found to be most abundant. From other areas *H. lineatum* is more prevalent. Comparable groups of animals indicated a prevalence of *Hypoderma* larvae in the esophageal region. Of 982 larvae removed from infested esophagi, 981 were *H. lineatum* and only 1 was *H. bovis*. Only the larvae of *H. bovis* occurred regularly in the spinal canal and only *H. lineatum* occurred regularly in the esophageal region.

H. Pyenson

Also see abs. no. 780.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

789. Production methods and keeping quality of churning cream. H. R. THORNTON, R. K. SHAW and F. W. WOOD. Can. Dairy Ice Cream J., 28, 5: 54-66. May, 1949.

Sterile utensils are necessary for the production of low bacterial content cream, as well as for low bacterial content of market milk. Special grade may be obtained with twice-weekly delivery when the storage temperature of cream of low bacterial content is 50° F. and with weekly delivery when the storage temp. is not over 45° F. The long storage of such creams at temp. of 50° F. or below probably will result in bacterially-induced flavor defects not now very common in the churning cream industry in Canada. In these circumstances the titratable acidity test may become of very limited value as a differential criterion in grading.

H. Pyenson

790. Continu boterbereiding volgens het Alfa-procédé. (Alfa continuous buttermaking process.) (English summary.) Algemeene Nederlandsche Zuivelbond. (General Netherlands Dairy Association.) The Hague, Holland. 30 pp. 1949.

Experience was obtained with apparatus and product of the Alfa continuous process. Cream of about 30% fat content was pasteurized, cooled to 55-60° C. and concentrated to plastic cream with butter composition by a "concentrator", a special Alfa-Laval centrifuge skimming to 0.07% fat. In a "transmutator" the plastic cream was converted into butter. The transmutator is a cooling unit having large diameter screws which transport the cream in a thin layer through three cooled cylinders. In the middle cylinder, usually, the change into butter occurs. Particulars for operating and cleaning are given. The capacity was 400-550 lb./hr. The water content of the butter can be regulated by the concentrator with a cream adjusting screw. Important factors were: skimmilk pressure, number of revolutions, kind of milk, fat content of the 30% cream, concentrating temperature and milk acidity.

The quality of sweet butter was very good; the keeping quality in cold storage and at room temperature was most satisfactory. The consistency was very firm and spreadable. The butter had a low air content and a fine uniform dispersion of the water. Less favorable results were obtained with the artificially flavored product. Alfa butter had a somewhat different quality than ordinary butter, shown both in taste and in structure.

The output was high because the non-fat dry solids content was high and the water content could be adjusted very accurately. The conclusion was drawn that the Alfa process offers promise.

A. F. Tamsma

791. Whey butter. LORNE SCHENCK. Can. Dairy Ice Cream J., 28, 8: 42, 76. Aug., 1949.

The same standards of sanitation should apply to whey handling equipment as to that used in handling milk and curd in cheese making. The cream should be pasteurized immediately after the separating. To produce a firm-bodied butter, the cream should be churned at a low temperature and with a reasonably high fat content. Over- and under-churning should be avoided. All the buttermilk must be drained out before salting and working the butter.

H. Pyenson

792. Butter cutter. W. GROCOFF. U. S. Patent 2,479,742. 4 claims. Aug. 23, 1949. Official Gaz. U. S. Pat. Office, 625, 4: 1018. 1949.

Butter is cut into rectangular shapes by being pressed through a flanged channel by means of a lever-operated pressure plate.

R. Whitaker

Also see abs. no. 773, 803, 813, 814, 815.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

793. A study of the ripening of Cheshire cheese. M. BARON, National Institute for Research in Dairying, University of Reading. Dairy Ind., 14, 7: 705-711. July, 1949.

Eleven Cheshire cheeses of varying quality were studied in detail by rheological and subjective testing during a 2-month ripening period in a well-controlled commercial cheese store (curing room). Despite the small number of samples involved in the experiment, it seems fairly clear that one can distinguish at a very early stage between cheeses of diverse future quality by several rheological tests. The variation of the mechanically measured properties of the cheese during ripening can also be used to give some indication of the type of cheese which is developing.

G. H. Watrous, Jr.

794. Further studies with lipolytic bacteria and rancid flavors in Cheddar cheese. E. G. HOOD, C. A. GIBSON and J. F. BOWEN. Can. Dairy Ice Cream J., 28, 6: 27-28, 82. June, 1949.

An analysis of the results of 11 comparative pairs of vats made from milk containing varying percentages of lipolytic bacteria shows consistently higher acid values and lower flavor scores than uninoculated or control vats. Further evidence is presented to show that the higher the acid value of the cheese fat, the greater is the incidence of unclean and rancid flavor defects. H. Pyenson

795. Basic research and the cheese industry. C. W. ABBOTT, Natal Agr. Research Institute, Pietermaritzburg. Farming in South Africa, 24, 279: 306-310. June, 1949.

Preliminary studies have shown that the yield of Gouda and Cheddar cheese per 1,000 lb. of milk is seasonal and follows closely the pattern of seasonal variation in the composition of milk from which it was made. The seasonal influence on Gouda is much more marked than on Cheddar.

F. C. Fountaine

796. Lack of mould growth in Roquefort cheese. S. BAKALOR, Agr. Research Institute, Pretoria. Farming in S. Africa, 24, 278: 246. May, 1949.

Mold growth in certain experimental lots of Roquefort cheese was poor or entirely lacking. The pH of such cheese ranged from 4.8 to 6.2 with most samples falling between pH 5 and 6. Cheese with satisfactory mold growth had pH values between 6.25 and 6.75. The author sug-

gests that acidity control should be practiced in the manufacture of Roquefort cheese.

F. C. Fountaine

797. Consumer preference as related to acidity, curd size, creaming. W. H. E. REM, Univ. of Missouri, Columbia. Proc. 41st Ann. Convention Milk Industry Foundation, Plant Sec., vol. 2, p. 21. 1948.

Consumer demand has developed to the point where cottage cheese manufacture must be regarded as a year-round business instead of merely an outlet for surplus milk during the flush period. Uniformity of product is of great importance in building and maintaining a cottage cheese business. Production of cottage cheese increased 250% in the last 14 yr. The product possesses high nutritional values, particularly as an economical source of protein.

"Pop corn" type curd largely has replaced the "smearcase" type of curd, although a few markets still manufacture both types. The optimum acidity of the curd at cutting is 0.57 to 0.63%, or if the whey is tested, 0.45 to 0.50%. The causes of mealiness, lumpiness, and a soft pasty texture are discussed.

Nonfat dry milk solids can be used in cottage cheese manufacture, starting with a product reconstituted in the proportion of 20 lb. nonfat dry milk solids to 80 lb. water. The proper acidity of the curd at cutting varies with the characteristics of the powder but the average value was 0.70%. Two advantages to the use of nonfat dry milk solids for cottage cheese are uniformity of product and availability of raw materials throughout the year to meet market demands for cottage cheese.

D. J. Hankinson

Also see abs. no. 810.

CONDENSED AND DRIED MILKS;
BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

798. Whole milk powder made with a minimum of heat treatment. U. S. ASHWORTH, Dept. of Dairy Husbandry, State College of Wash., Pullman. Milk Plant Monthly, 38, 8: 68-70. Aug., 1949.

The effect of heat on milk and its relation to the keeping quality of whole milk powder is divided into 3 categories: destruction of enzymes, production of antioxidants and reduction of bacterial contamination. Using a review of literature in conjunction with personal observations, the author presents evidence for and against preheating of milk to a high temp. prior to condensing and drying.

J. A. Meiser, Jr.

799. 6-8-6 Formula produces higher quality bread. C. A. GLABAU, Bakers Weekly Lab., New York, N. Y. Bakers Weekly, 143, 9: 34. 1949.

A comparison was made of properties of 2-4-2, 4-6-4, 6-6-6 and 6-8-6 doughs, and breads (Ratios as % M.S.N.F., sugar and shortening, respectively). A complete table of values shows breads of higher percentage ingredients have very favorable properties. K. G. Weckel

800. Milk—Its contribution to improved bread quality. C. A. GLABAU. Bakers Weekly, 143, 11: 36-40. Sept. 12, 1949.

Using a 6-8-6 basic ingredient formula, representing the percentages of non-fat dry milk solids, sugar and shortenings, respectively, experimental breads produced from doughs wherein the M.S.N.F. was varied from 2 to 6% were examined. The study showed increased wt. of dough, bread yield, softness and brownness of color, and reduced baking and cooling loss, and resistance to shear when M.S.N.F. was increased from the lowest level. A complete comparative table is provided. K. G. Weckel

801. Consumer reaction to bottled fresh concentrated milk. G. M. TROUT and G. G. QUACKENBUSH. Can. Dairy Ice Cream J., 28, 7: 68-74, 82. July, 1949.

Bottled fresh concentrated milk concentrated at ratios of 2:1 and 3:1, homogenized and pasteurized, was furnished consumers in order to find out their reactions to the product. Although low temperatures in forewarming were used to keep the formation of heated flavors to a minimum, the cooked type of flavor seemed to predominate in the reconstituted product. Homogenization pressure of 2,000 to 2,500 lb./in.² were adequate to maintain satisfactory homogeneity of the reconstituted product. The type of water used was found to be a factor affecting the flavor of the reconstituted milk. The predominating cooked flavor was not objectionable to the majority of the consumers surveyed in the study. The consumers were not interested in buying it regularly unless a saving of 2 to 3¢/qt. of milk equivalent could be effected. H. Pyenson

802. Method of canning evaporated milk and similar foamy liquids. G. G. GRINDROD. U. S. Patent 2,477,692. 8 claims. Aug. 2, 1949. Official Gaz. U. S. Pat. Office, 625, 1: 123. 1949.

To avoid foaming which occurs frequently when evaporated milk is filled into vent-hole type cans, the product is filled hot into the cans which have been deaerated by filling with steam. R. Whitaker

803. Plastic cream and butter oil. C. W. DECKER. Can. Dairy Ice Cream J., 28, 8: 68-76. Aug., 1949.

The article reviews the manufacture of plastic cream and butter oil separately, discussing their development, processing methods and equipment employed, uses, keeping qualities and future possibilities. H. Pyenson

804. Dry egg composition. E. K. CHAPIN. U. S. Patent 2,479,310. 2 claims. Aug. 16, 1949. Official Gaz. U. S. Pat. Office, 625, 3: 775. 1949.

A spray dried egg product having good keeping quality, improved usefulness in baked goods and desirable dispersibility is described. The liquid egg, prior to drying, is mixed with either liquid skim milk or whole milk and an edible fat.

R. Whitaker

805. Preparation of high-grade crude lactose. E. F. ALMY and O. F. GARRETT. (Assignors to Mand R. Dietetic Laboratories, Inc.) U. S. Patent 2,477,558. 8 claims. Aug. 2, 1949. Official Gaz. U. S. Pat. Office, 625, 1: 92. 1949.

Lactose solutions, prepared from whey or skim milk, are treated with cationic exchange material in a pH range of 4.6-4.8. The proteins are pptd. by heating to 175-210° F. and removed from the lactose solution. The solution is then demineralized at pH 7.5-9.0 in an anionic exchanger, acidified to 6.5-6.8 and dried.

R. Whitaker

Also see abs. no. 876, 877.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

806. Comparative analysis of the standard methods methylene blue stain and advantages of the polychrome and acid-and-water-free stains in the direct microscopic examinations of milk. S. B. LEVINE and L. A. BLACK, U. S. Pub. Health Service, Cincinnati, Ohio. Am. J. Pub. Health, 39, 9: 1110-1119. Sept., 1949.

The two methylene blue staining solutions recommended in the 9th ed. of Standard Methods (p. 116) differ in both surface tension and dye concentration. Unless the retention of both staining solutions is justified by a comparative study, only 1 should be designated as standard; perhaps the alcohol-containing formula should be given preference. Partial decolorizing of milk films after staining with carbolated methylene blue resulted in counts significantly different (usually higher) than those obtained from the

"undestained" slide. The acid-and-water-free stain yielded the highest counts of all, while the polychrome methylene blue used in this study gave the lowest counts, results with carbolated methylene blue being intermediate. In a previous study, another batch of the polychrome stain had given higher results than carbolated methylene blue. No overstaining occurred with either batch of polychrome stain and the slides were more easily read than those stained by the carbolated methylene blue. Counts of dried milk films stained with the acid-and-water-free stain after 90 d. of storage were not significantly different than those counts obtained after 14 d. of storage.

D. D. Deane

807. Some factors which influence the thermoduric colony counts of raw milk. S. B. THOMAS, National Agr. Advisory Service, Trawscold, Aberystwyth. Dairy Ind., 14, 6: 586-589. June, 1949.

No appreciable difference was found in the thermoduric bacterial content of milk produced by hand milking or machine milking on farms where the utensils were efficiently sterilized by steam, boiling water or chlorine.

When the equipment was only washed in warm water, the use of milking machines led to a much higher incidence of excessive thermoduric colony counts.

The proportion of samples with high thermoduric colony counts increased with increasing raw milk counts, though some high count milks had low thermoduric counts.

There was also some association between grading on the routine resazurin test and thermoduric bacterial content.

Thermoduric colony counts on yeastrel milk agar incubated for 4 days at 30° C. were, on the average, 12 times those obtained on yeastrel milk agar incubated for 2 days at 37° C.

G. H. Watrous, Jr.

808. Controlling thermodurics. I. E. PARKIN, Penn. State College. Milk Dealer, 38, 10: 64-66. July, 1949; Milk Plant Monthly, 38, 7: 66, 68. July, 1949.

Thermoduric bacteria are discussed as to source and how they get into milk. Eradication may be accomplished by clean methods, clean cows, properly washed and sanitized equipment and managed milking. The latter consists of cleaning the udder, using the strip cup, eliminating hand stripping if machine milking is used, keeping the milk covered and properly cooling and storing the milk until it is shipped. Thermoduric organisms are resistant to heat and sanitizing agents. Rinsing of milk machine equipment

with cold water followed by a hot water rinse after milking therefore is an extremely poor procedure. A method for cleaning and sanitizing milking machines is outlined.

C. J. Babcock

809. The morphological and cultural characters of the lactic acidoproteolytic cocci. C. GORINI, Univ. of Milan. Enzymologia XIII, 4: 205-207. 1949.

Characteristics which are considered significant in the differentiation of members of this group of organisms are summarized. The shape of the curd appearing in a litmus milk culture is stressed, and a plate of descriptive diagrams of the curd appearing in typical milk cultures is presented.

J. J. Jezeski

810. The effect of penicillin on lactic streptococci. G. J. E. HUNTER, The Dairy Research Institute (N.Z.), Palmerston North, New Zealand. J. Dairy Research, 16, 1: 39-45. Jan., 1949.

The inhibitory effect of penicillin on 10 "single" strains of starter streptococci used in cheese manufacture was studied. *Streptococcus cremoris* strains were inhibited markedly by as little as 0.10 unit of penicillin / ml. of milk. *Streptococcus lactis* strains were not inhibited to the same degree unless 0.25-0.30 unit/ml. was present.

Very little destruction of penicillin occurred in milk pasteurized at 145° F. for 30 min. Steaming the milk for a period of 1 hr. resulted in approximately 50% loss.

The conclusion is that widespread uncontrolled use of penicillin in dairy herds could bring about a real problem in the cheese industry. It would seem advisable to exclude from the milk supply milk from treated quarters.

E. L. Thomas

811. Coliform—their significance and control in ice cream making. G. W. SHADWICK, Beatrice Foods Co., Chicago, Ill. Ice Cream Trade J., 45, 8: 44, 87-89. Aug., 1949.

Proper pasteurization of the ice cream mix, thorough cleaning and sanitizing of the equipment, care in handling ingredients and packages, and extreme cleanliness on the part of the worker are necessary if coliform-free products are to be produced.

Coliform bacteria are Gram-negative, non-spore-forming bacteria which ferment lactose with the production of gas. They grow equally well in the presence or absence of air. Since pasteurization destroys coliform bacteria, their presence in dairy products usually indicates recontamination or careless handling and does not necessarily indicate a health hazard, as they are

not pathogenic. Samples taken at various points throughout the plant during processing usually will indicate the point of contamination and make it possible to eliminate the cause of the trouble.

W. H. Martin

812. The average plate count ratio as a measure with which to judge laboratory work in examining dairy products. J. L. COURTNEY, Dept. of Public Health, Oak Ridge, Tenn. J. Milk and Food Technol., 12, 4: 197-208. July-Aug., 1949.

A study was made of the av. quantitative plate count on 7,427 samples of raw milk, pasteurized milk, cream, frozen desserts and chocolate beverage at Oak Ridge, Tenn., over a period of 14 mo. An average was determined of the count ratios of those samples for which both dilutions show a range between 30 and 300 colonies. This ratio should not be over 2.0, according to the Standard Milk Ordinance and Code. The average was used as a measure to check the accuracy of the laboratory work.

The av. standard plate count ratio of 2.31 was reduced to 1.85 by minimizing the errors common to pipetting. A carry over of 1 drop on the end of the pipette during plating will contribute to a high average ratio. High average ratios are due primarily to careless laboratory workers and the failure of sanitary officials to use the average as a check on the quality of work done in the laboratory. H. H. Weiser

813. Some observations on bacterial discoloration of butter. A. G. LEGGATT. Can. Dairy Ice Cream J., 28, 7: 29-30. July, 1949.

In the 4 cases given it was shown that the trouble was due to carelessness in some part of general plant sanitation. The butter can be infected by 90 lb. butter boxes, parchment wrappers or wooden ware. Contrary to current belief, this discoloration occurred in butter salted at the rate of 2%. H. Pyenson

814. The nature and quantity of fatty acids produced in butterfat by the action of microorganisms. T. RICHARDS and G. M. EL-SADEK, University of Reading, Reading, England. J. Dairy Research, 16, 1: 46-52. Jan., 1949.

The fatty acids were extracted from 12 samples of rancid butter and fractionated into volatile, solid and liquid groups according to the method of Hilditch. Three samples of butter were made rancid with inoculations of pure cultures of bacteria (*Achromobacterium*, *Micrococcus* and *Pseudomonas* species) and 3 with pure mold

cultures (*Aspergillus*, *Cladosporium* and *Penicillium* species). Six others were obtained in a naturally rancid condition and various species of molds and bacteria were isolated and identified for comparison. The total amounts of acids recovered varied from 0.2 to 1.05% of the total weight of butterfat. In every case the mold samples produced a greater total of fatty acids than did bacteria under identical conditions. The volatile acids constituted from 3.5 to 8.2%, the solid group from 4.3 to 11.2% and the liquid acids from 77 to 85% of the total acids extracted. Bacteria produced relatively less volatile acids than molds but slightly more solid acids. Titration values indicated that bacteria produced a greater proportion of butyric and caproic acids than did molds. No significant difference between bacteria and molds was noted in the production of the liquid unsaturated acids. Iodine values and titration equivalents of the liquid acids indicated a high proportion of oleic and linoleic acids. The suggestion is made that triolein or trilinolein might prove more reliable than tributyrin as a substrate in a medium for the detection of lipolytic bacteria, since these acids appear to make up such a large fraction of the acids recovered from rancid butter.

E. L. Thomas

815. A method for the bacteriological examination of edible fat preparations. B. F. CAPPS, M. K. WOLLMAN and N. L. HOBBS. R. P. Scherer Corp., Detroit 13, Mich. Food Tech., 3, 8: 260-263. Aug., 1949.

Standard plate counts on contaminated oil resulted in a dense cluster of colonies around the oil droplets formed on the surface of the agar. In order to eliminate this difficulty, various detergents were tested for their effectiveness in dispersing oil in an aqueous solution to be used in a standard plate count examination. Ten ml. quantities of the aqueous detergent solution of specified concentration were autoclaved in culture tubes with cotton plugs. One ml. of the contaminated oil was added to each tube of detergent solution and the pipette used to mix the inoculum to form an emulsion. The emulsion then was transferred in 1 ml. aliquots to plates and tube media; the plates were poured with the agar medium and after incubation the colonies were counted.

Of the detergents tested, the Tweens showed the greatest advantages. The optimum concentrations were found to be 2% by volume of Tween 80, and 3% by volume of G-2800 in aqueous solution to produce maximum emulsification of the oil with the least unfavorable results.

•E. R. Garrison

816. Inactivation of bacteriophage of the lactic acid streptococci of starters by quaternary ammonium compounds. C. C. PROUTY, Wash. Agr. Expt. Sta., Pullman, Wash. *J. Milk and Food Technol.*, 12, 4: 214-218. July-Aug., 1949.

Several quaternary ammonium compounds were effective in the inactivation of bacteriophage. Alkyl di-methyl benzyl ammonium chloride did not inactivate the bacteriophage when used in 100 p.p.m. at 2 and 4 min. exposure periods, although this compound was more effective at lower concentrations than several of the other preparations. N (acyl colamino formyl methyl) pyridium chloride was the least effective in the low concentrations. Based upon U. S. Public Health Service recommendations for Cl_2 solution, a concentration of 200 p.p.m. of a quaternary ammonium compound for a 2-min. exposure should destroy the bacteriophage of the lactic acid streptococci used in cheese starters. H. H. Weiser

817. Pasteurization effect on bacteria, yeast, molds and enzymes. P. R. ELLIKER. *Can. Dairy Ice Cream J.*, 28, 6: 50-60, 88. June, 1949.

The article discusses methods of pasteurizing, time-temperature relationships required for bacterial destruction, effect of pasteurization on various microorganisms in milk and milk products, disease producing bacteria, common lactic acid bacteria, coliform bacteria, ropy milk bacteria, water bacteria, spore formers, molds and yeasts, bacteriophage and antibiotics in cheese milk and effect of pasteurization on enzymes of milk and milk products. H. Pyenson

Also see abs. no. 774, 779, 780, 784, 785, 786, 789, 794, 796, 869.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

818. A rapid and simple phosphatase test for milk. R. ASCHAFFENBURG and J. E. C. MULLEN, National Institute for Research in Dairying, Reading, England. *J. Dairy Research*, 16, 1: 58-67. Jan., 1949.

The test is based on the use of p-nitrophenyl-phosphate as the substrate. The milk is incubated at 37° C. with a buffered solution of the substrate, followed directly by a visual comparison of the intensity of yellow color of liberated p-nitrophenol with that of boiled milk standards containing known amounts of p-nitrophenol. The presence of 0.2% raw milk could be detected without fail after an incubation period of only

30 min. Upon incubation for 2 hr. the test was sensitive to the presence of 0.1% raw milk. The test was proved sensitive to a drop of 1° F. in pasteurizing temperature (143° F.). Detailed information is given concerning reagents and apparatus required for the test and for the preparation of color standards. Instructions for reading the test by photoelectric colorimetry also are included. E. L. Thomas

819. A modified Association-Babcock test for homogenized milk. J. R. BRUNNER, G. M. TROUT and P. S. LUCAS, Dept. of Dairying, Mich. State College, East Lansing. *Milk Plant Monthly*, 38, 8: 45-46, 48. Aug., 1949.

Since numerous modifications of the Babcock method for testing homogenized milk do not give reliable tests, experiments were conducted to adapt the I.A.M.D. modified Babcock test for buttermilk to the testing of homogenized milk. The testing procedure as finally adopted differed from the original Association test in (a) tempering of the milk and reagents to 60° F., (b) use of 18 g. of milk, (c) addition of 3 ml. of n-butyl alcohol and 14 to 16 ml. of commercial sulfuric acid and (d) use of glymol in reading the test. This procedure gave a clear, curd-free fat column that averaged within +0.05% of the Mojonnier method. J. A. Meiser, Jr.

820. Determination of lactose in milk products. B. D. HITES, C. W. ACKERSON, and G. H. VOLKMER, Agr. Expt. Sta., Lincoln, Neb. *Analyt. Chem.*, 21, 8: 993-995. Aug., 1949.

Lactose and sucrose were determined in dairy products by the ferricyanide method, which was shown to be simple, convenient, and time-saving. The method was used for the analysis of dairy products containing lactose and lactose in the presence of sucrose. It cannot be used when the products contain other reducing sugars. Standard lactose and sucrose curves and curves for sucrose plus lactose are presented. A comparison of values obtained by the ferricyanide and copper reduction methods on lactose recovered from whole milk and other common dairy products shows close agreement. B. H. Webb

821. A conductometric method for the determination of ash in refined lactose. D. A. BREWSTER and BARBARA A. BREWSTER. *Food Tech.*, 3, 6: 208-210. 1949.

The conductance method of Zerban and Statler was adapted so that it could be used for refined lactose. The method outlined is limited to lactose that contains between 0.001 and 0.200% ash. The electrical method is 18% more accurate on

the average than the muffle procedure and is much more rapid and easily performed.

E. R. Garrison

822. The prooxidant effect of ascorbic acid and cysteine in aqueous fat systems. DOROTHY A. SCARBOROUGH and BETTY M. WATTS. *Food Tech.*, 3, 5: 152-155. 1949.

A new system for testing antioxidants and synergists in aqueous fat systems was employed. In the absence of added phenolic antioxidants, aqueous solutions of ascorbic acid or cysteine accelerated the oxidation of lard but the accelerating effect of ascorbic acid was not obtained on dry fat. Ascorbic acid inhibited oxidation in aqueous fat systems when 0.01% or more of α -tocopherol was added.

E. R. Garrison

823. Denaturation in regenerated protein fibres. F. HAPPY. *Nature*, 164, 4161: 184. 1949.

By means of X-ray photographs it is shown that the casein in artificially produced fibres is of the β and not of the α type.

R. Whitaker

Also see abs. no. 773, 794, 881, 882.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

824. Mechanical can washing. C. B. SHOGREN. *Can. Dairy Ice Cream J.*, 28, 5: 98-100. May, 1949.

The rules that should be observed in operating any type of straight-line can washer are: (1) keep the washer in a clean, sanitary condition free from lime and deposit, (2) keep the washer in good mechanical condition, (3) an adequate pre-rinse must be maintained with ample water supply and a good line pressure of at least 30-40 lb., (4) fresh solution must be made up daily and sufficient washing compound used to form a heavy foam cap on the wash tank, (5) overflow should be controlled at between 0.5 and 1 U. S. pt./can, (6) treatment should be made to the sterile rinse position to keep pipes and jets in lime-free and clean condition.

H. Pyenson

825. Lifting device. F. G. HODSDON. (Assignor to International Harvester Co.) U. S. Patent, 2,480,244. 5 claims. Aug. 30, 1949. *Official Gaz. U. S. Pat. Office*, 625, 5: 1278. 1949.

Milk cans are lifted into and out of milk coolers by means of this device which consists of a vacuum operated reciprocating piston and cylinder and hooks for attaching to the can handles, the

whole being supported by a single pipe vertical frame bolted to both floor and ceiling.

R. Whitaker

826. Balances milk-chilling water flow by raising supply tank. M. N. KRAUSE. *Power*, 93, 8: 92-4. Aug., 1949.

A setup for cooling with chilled water circulated through milk and by-products units did not give satisfactory cooling because of an unbalanced flow through the various heat exchangers. The system was changed by installing an insulated supply tank on the 4th floor and new booster pumps. Both manual and automatic controls were installed to operate the pumps. The remodeling made use of the existing circulating pumps and mains wherever possible. The installation was designed and carried through with very little interruption of processing. Graphs of tests and diagrams of the piping and control wiring are presented.

H. L. Mitten, Jr.

827. Milk can cooler. R. D. KEMPER. (Assignor to York Corp.) U. S. Patent 2,479,011. 5 claims. Aug. 16, 1949. *Official Gaz. U. S. Pat. Office*, 625, 3: 699. 1949.

Milk in regular milk cans is cooled by placing the cans on racks in this insulated cabinet. Water cooled by a motor driven compressor, set to automatically cool the water to near the freezing point, is sprayed on the cans from distributor pipes. The water collects in a pump below the racks and is circulated back to the cooling unit.

R. Whitaker

828. Machine for cooling cream. A. JOHNSON. (Assignor to Land O'Lakes Creameries, Inc.) U. S. Patent 2,480,583. 3 claims. Aug. 30, 1949. *Official Gaz. U. S. Pat. Office*, 625, 5: 1364. 1949.

Cream is cooled by spreading it in a film on horizontally rotating, internally chilled rolls.

R. Whitaker

829. Water-cooling tower upkeep. H. E. DEGLER. *Operating Engineer*, 2, 8: 36-7. Aug., 1949.

Makeup water required depends upon losses from evaporation, drift and blowdown. Blowdown water wasted depends upon the hardness of the circulating water, type of softening used and the drift loss. Blowdown is controlled to keep scale-forming solids at such a low concentration that scale does not form.

Additions of Cl_2 , CuSO_4 , KMnO_4 and other chemicals will hold or eliminate algae growth so that such formations will not plug nozzles. The

pH of the circulating water should be 7 to 7.5 to prevent delignification of any wooden parts of the tower contacting water. Two-speed motors on the fans of the induced draft towers save power during cold weather or other times when full speed is not required to obtain temperature lowering.

Maintenance consists of seeing that the tower is clean, dirt is removed from catch basin beneath tower, fans are bolted tightly, blades run freely, flexible couplings and universal joints are functioning properly and fans and fan motors are properly lubricated. The catch basin float valve should be adjusted to keep the water level 5 to 6 in. below wood filling. At least 6 in. water should be kept in redwood or steel basins. Nozzles must be kept free of scale and algae. Operation during cold weather requires reduced draft. Icing during periods of sub-freezing weather may be prevented by keeping the water temperature as high as practicable for the equipment served.

A maintenance schedule should be set up and followed.
H. L. Mitten, Jr.

830. Refrigerating your ice cream fleet. Anonymous. *Ice Cream Rev.*, 33, 1: 48, 50, 52, 53. Aug., 1949.

Truck bodies with a plate type hold-over system account for more than 90% of the current orders, according to results of a survey conducted among the leading manufacturers of this equipment. Over two-thirds of the truck bodies being purchased also have self contained refrigeration units which may be plugged in at an electric outlet wherever the truck may be stationed at night. The practice of using a central ammonia system for supplying the necessary refrigeration for the hold-over plates appears to be in the decline, as only one-third or less of the current orders are for this type of truck body.

Dry ice which was used in about one-third of the ice cream truck bodies 10 yr. ago is now used in less than 2% of them. The use of dry ice for refrigerating ice cream truck bodies has proved to be very expensive in comparison with mechanical refrigeration. Its use is now limited primarily to semi-trailer type trucks making few stops and to vendor units.

A power take-off for supplying continuous refrigeration to ice cream truck bodies is used by only about 1% of the trucks, according to 1 manufacturer.
W. J. Caulfield

831. Refrigerated units for trucks is cost reducer. JOHN HUBEL. *Am. Milk Rev.*, 11, 9: 36, 37. 1949.

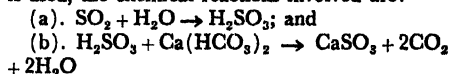
A method used by 1 milk plant is described. Retail delivery trucks are equipped with a re-

frigerated cabinet of 36-case capacity. The method of refrigeration is not stated. Advantages claimed for these cabinets are (1) trucks are preloaded and ready for the routeman and (2) unsold products are left on the truck and sold the next day, thus eliminating handling and checking in.
D. J. Hankinson

832. Liquid sulphur dioxide stops scale in cooling-water systems. M. E. REINER, E. F. Drew & Co., Inc., 15 E. 26th St., New York City. *Power*, 93, 8: 86-7. Aug., 1949.

Sulphuric acid can be used to treat industrial cooling water where pH and alkalinity must be lowered. This acid is difficult and hazardous to handle. Sulphur dioxide may be substituted fairly inexpensively. It can be injected as a gas into the makeup water.

Impurities in water are concentrated by the evaporation of water in cooling equipment. Scale is caused by the breakdown of CaCO_3 . When SO_2 is used, the chemical reactions involved are:



Use of SO_2 is limited to those applications where makeup is moderate, for its cost is greater than either sulphur burner gas or H_2SO_4 . It is the logical choice where its higher cost is more than balanced by reduced hazards and ease of handling.

Drawings illustrate piping hook-ups for injection, and a table compares costs. H. L. Mitten, Jr.

833. What plant operators need to know about flue dust. L. N. ROWLEY and J. C. McCABE. *Power*, 93, 8: 76-8. Aug., 1949.

Dust differs from smoke in that individual particles of it are large enough to be seen and felt. Most fuels contain some ash. Gas has practically none, oil very little and coal, rarely, has less than 5% and may carry as much as 20-30%. Regardless of how good the burning job is, ash always remains. Some unburned carbon usually accompanies the ash to make flyash or cinder. Flyash particles are smaller than cinder particles; both may be called dust. Dust production cannot be avoided; however, dust scattering and air-pollution from smoke stacks can be eliminated.

With fuel-bed firing by underfeed or traveling grate stokers, the airflow through the fuel-bed carries out the dust. Heavier drafts lift more dust from the bed into the gas stream. Coal plays an important part, since fines increase the carryover and clinkering tends to reduce it. High burning rates also cause more dust carryover, because

vid burning requires more draft. Pulverized with suspension firing presents the most serious flyash problems.

Ordinances usually state the maximum amount of dust to be tolerated in a given amount of flue gas without regard to the amount of air pollution. Sample calculations show that some form of collector is necessary. Seven different types of dust collectors are illustrated. They employ such factors as change of gas velocity, sudden change in direction of gas flow, impingement, centrifugal force, electrostatic precipitation and water washing.

Chimneys can aid in reducing air pollution, for the higher the stack, the greater is the dust travel and diffusion. This is affected by such factors as dust size, local weather conditions and surrounding topography. Chimneys are as much waste-disposal equipment as draft producers.

H. L. Mitten, Jr.

834. What to look for in a package boiler. J. F. JOHNSTON, Johnston Bros., Inc., Ferrysburg, Mich. Operating Engineer, 2, 8: 24-5. Aug., 1949.

Packaged boilers are automatic units complete with boiler, auxiliaries, controls and instruments. They are assembled and tested at the factory and shipped ready for simple installation.

Advantages are that no special setting is required, no smokestack is necessary, and the complete unit is sold, guaranteed and serviced by a single firm.

Before selecting a packaged boiler determine the actual h. p. load and the hours of use. Packaged unit capacity is rated on the basis of maximum amount of steam that can be generated allowing 34.5 lb. (from and at 212° F.) / boiler h. p. It is safer to buy a packaged unit that can develop your maximum load in lb. of steam / hr. plus a little extra.

Special burners are necessary to burn heavy oil, but heavy oil is lower in cost and has a higher BTU content.

The design of packaged units varies considerably. A careful study of design features and auxiliaries should be made if one is to select a unit that will meet all his demands. The unit must be approved by such organizations as Underwriters Laboratories on electrical devices, National Electrical Code on wiring, ASME code on boiler construction.

A packaged unit requires proper maintenance. There should be a regular periodic interior inspection. Scale and sediment must not be permitted to accumulate. Controls and safety devices should be tested and natural wear and tear corrected.

H. L. Mitten, Jr.

835. How heating fits into steam costs. W. SHINN and W. ARROTT, Operating Engineer, New York, N. Y. Operating Engineer, 2, 8: 27. Aug., 1949.

An illustrative problem is presented and discussed. The problem plant considers heating as a by-product of other steam requirements.

H. L. Mitten, Jr.

836. Here's how to cut piping operating costs. G. W. HAUCK, Crane Co., Chicago, Ill. Heating, Piping & Air Conditioning, 21, 8: 90-2. Aug., 1949.

Good design places valves in the most convenient locations for operation, but some valves must necessarily be placed where they are relatively inaccessible from the normal level of operators. The amount of time which must be spent in getting to the valve may cumulate costs which need challenging. Valve stem extensions, wheel chains and motor operated valves may effect economies in operational labor. Position indicators eliminate the need for turning the valve stem to determine whether the valve is open or closed. One of the typical ills of the improvised piping installed during recent years is misapplication of valves. Now that correct types are available, valves not properly fitted to the application cannot be justified.

Another common fault is improper suspension of piping. Weight of pipes and fluid can strain joints to the point of leakage, distort valve seats or contribute to maintenance costs in other ways.

H. L. Mitten, Jr.

837. Pumps need to be piped up right. H. M. SPRING, Canton, Mass. Operating Engineer, 2, 8: 38-39. Aug., 1949.

Piping must be of correct strength for the pressure it will carry. If the shutoff pressure of the pumps exceeds the safe pressure for the discharge pipe, a relief valve is needed. Its discharge should be sufficient to keep the pressure within the safe range.

Where there is a negative suction, an undersized pipe or restriction in the suction pipe can make it impossible for the pump to develop rated capacity. For high-suction lifts, piping one size larger than pump connection should be used. In this case an eccentric reducer should be used to make the connection. The piping layout should be made so that there is no chance for air pockets. Pitch of suction lines should be away from the pump. Temperature of the fluid being pumped affects the effective suction. The higher the temp., the lower the effective suction.

Where there is a positive suction, maintenance is made easier if a valve is installed near the pump. Sharp turns in discharge lines should be avoided with reciprocating pumps. Where hot liquids are handled there must be provision for pipe expansion. Priming of centrifugal pumps for suction lifts may be made with a priming line with shut-off valve to the highest part of the pump casing.

H. L. Mitten, Jr.

838. Making metal corrosion-resistant. L. SANDERSON. *Dairy Ind.*, 14, 7: 721-723. July, 1949.

Zincilating, one of the newer methods of making metals corrosion-resistant, can be applied to parts during their manufacture or in service, to water tanks, liquid tanks, containers, etc. by spraying with a gun, by dipping, or with a brush.

Another method of making metal corrosion-resistant is phosphatizing. In this, Fe, steel, Zn, Al, Cd and their alloys are treated by immersion in a dilute solution of acid phosphate saturated with 1 or more of these metals. This converts the metallic surface into an insoluble crystalline phosphate coating, which is protective and integral with the metallic surface. Phosphate coatings are advantageous when it is desired to produce coatings that withstand corrosion and, also, provide a satisfactory base for paint.

Non-hard drying and hard-drying rust-preventing compounds that are applied to steel to safeguard it against great extremes of temperatures and moisture content in the air are briefly discussed.

G. H. Watrous, Jr.

839. Fast check for shaft alignment. E. MARSELLI, St. Louis, Mo. *Operating Engineer*, 2, 8: 32-3. Aug., 1949.

Flexible couplings should be checked periodically for misalignment. Usually, it is necessary to uncouple the parts for these tests. A simple device is described and illustrated which permits the coupling to remain intact and cuts the checking time to around 10 min. The essential parts are 2 clamps, an indicator rod and a dial indicator. Methods are discussed for checking parallel and angular misalignment with the device.

H. L. Mitten, Jr.

Also see abs. no. 790, 851, 867, 872, 883.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

840. Cost system for ice cream manufacturers. JAMES H. GOULD, Business Engineering Council, Rochester, N. Y. *Ice Cream Rev.*, 33, 1: 39-40, 56-59. Aug., 1949.

A simplified cost accounting system for use in dairy plants is presented and discussed. It is designed to supply management with the essential facts and figures necessary for efficient operation of the business with a minimum of clerical help. Included in the management report are: (1) A monthly summary of total and unit cost figures to make, or to make and sell each product, (2) A plant expense budget showing a comparison of actual expenditures for each account with calculated budget allowance for that account and (3) A profit and loss statement showing the accumulative profit and loss by products for the current accounting period ending with the current month. The latter report also shows variances in the use of raw materials, in the use of labor, in plant expenses and in selling and administrative expenses.

Any plant interested in a simple but comprehensive cost accounting system will find the forms which accompany the article very useful in setting up such a system.

W. J. Caulfield

841. Industry consumer survey provides data for increasing ice cream sales. Anonymous. *Ice Cream Rev.*, 33, 1: 46, 74, 75. Aug., 1949; *Ice Cream Trade J.*, 45, 8: 32-34, 85-87. Aug., 1949.

In an effort to obtain information which might aid in increasing the sale of ice cream a survey is being conducted by the Am. Dairy Assoc. The present article deals with the preliminary results obtained to date.

It was determined that appetite appeals are the most effective means of advertising ice cream. Refreshing, delicious, variety of flavor, cool and appetizing illustrations were the appeals thought to be most effective. Health and food value appeals were thought to be important by the manufacturers interviewed but only 31 out of 160 dealers considered this type of appeal effective. Quality was thought to be an effective sales appeal by 30 of the 160 dealers interviewed but not 1 of the manufacturers interviewed mentioned this point. Economy appeals were thought to be more effective by dealers than by the manufacturers.

Point-of-sale advertising was favored by 34 of 50 dealers interviewed. Window banners were rated as the most effective type of point-of-sale advertising followed in order by back bar posters, complete bar trim, menu clips-ons, counter cards and over-wire hangers.

Almost half (42.8%) of 1073 families interviewed served ice cream in the home as a dessert. This held true whether there were children in the family or not.

Ice cream as a snack for family members was served by over one-third of the families inter-

viewed. Ice cream was served at parties by 11.9% of the families and on other occasions by 0.7% of all families. Considerable variation between different cities was observed as to the occasions on which ice cream was served.

The use of ice cream in the home by days of the week was found to be as follows: 29.0% of the families interviewed served ice cream on Sat., 22.5% on Fri., 13.3% on Thur., 15.9% on Wed., 12.2% on Tue. and only 5.5% on Mon. Most purchases of ice cream were made between the hours of 4 and 8 p.m.

The housewife made 45.1% of the ice cream purchases, the husband 19.9, a daughter 12.5, a son 11.4, children 4.4 and all others 6.7%.

The place from which ice cream was purchased for family use was found to be as follows: drug store 31.4, grocery 27.7, confectionary 12.5, delicatessen 12.5, ice cream store 10.7, dairy 1.8 and all others 3.4%.

The type of store where ice cream was purchased was selected by 47.4% because of convenience, by 33.3% because of brand preference and 18.9% because of a combination of these 2 factors.

W. J. Caulfield

842. Building gallonage through restaurants. VINCENT M. RABUFFO. *Ice Cream Trade J.*, 45, 8: 28-29, 77-78. Aug., 1949.

In a recent survey 11,000 customers in 138 restaurants were asked to rate ice cream as a dessert. Ice cream was rated most popular, pie a close second, and pie plus ice cream rated almost as high as ice cream and pie individually. Three-fourths of the vote went to these 3 desserts and only one-fourth to all other desserts combined. Sales portfolios prepared for use by restaurant operators point out the advantages of ice cream over other desserts. In addition to being lower in cost, ice cream is ready to serve, always fresh and always available; there is no waste and no left-over problem. At \$1.80/gal., using a no. 16 scoop to dip 28.3 servings, the per portion cost is 0.0633¢, compared to 0.0679 for pie and 0.0643 for cake. With some 525,000 eating places in the U. S., the ice cream industry has an opportunity to step up sales through these outlets.

W. H. Martin

843. Possible economies in distribution and sale of fluid milk. G. M. CARLYLE. *Can. Dairy Ice Cream J.*, 28, 6: 32-41, 84. June, 1949.

The following panel discussions were given at the annual meeting of the National Dairy Council of Canada: (1) Every-other-day delivery, by W. F. Jones, (2) Three-day delivery, by E. G. Silverwood, (3) Store differential, by E. A. Lewis, (4)

Other possible economies, by C. E. McMonagle, (5) Tokens, by G. M. Carlyle. H. Pyenson

844. Business charts an aid to milk plant management. F. MERISH. *Milk Plant Monthly*, 38, 8: 34-36. Aug., 1949.

Accounting serves both mathematical and statistical purposes, but obtaining statistical information out of columns is a difficult task. Graphs, however, present information in a clear concise manner which can be visualized readily. The several types of graphs available are the pie chart, bar diagram and the line graph. Milk plant operators, both large and small, should use this means of gauging business profitability and managerial ability for themselves and the employees as well.

J. A. Meiser, Jr.

845. Keeping costs down. D. MARKSTEIN. *Milk Plant Monthly*, 38, 8: 58, 61, 65. Aug., 1949.

Interviews with 6 milk plants in New Orleans showed that their methods of holding down costs followed 2 patterns: namely, increasing sales by intensive selling campaigns, reducing small costs and paying close attention to overhead and operating expenses.

J. A. Meiser, Jr.

846. Rewarding routemen for steady sales gains. T. KNIGHT. *Milk Plant Monthly*, 38, 8: 40-41. Aug., 1949.

Each routeman receives a certain bonus for each new qt. of milk over the previous base and this sum is increased each mo. if they keep adding business. Failure to increase sales during 1 mo. automatically causes the routeman to start over and at the lowest bonus rate.

J. A. Meiser, Jr.

847. Giving added push to sales drives. K. STRONG. *Milk Plant Monthly*, 38, 7: 69, 75. July, 1949.

In a drive to get all their customers to use homogenized milk, this plant decided to turn over the 1¢ differential to each routeman who started a customer on homogenized milk and maintained this for 20 d. Progress of this new customer drive was recorded on a large map of the U. S.

Each driver needed 15 extra quarts to ride a paper bull pinned on the map and as his sales increased the animal moved from East to West until the end of the 6-wk. contest. In a later contest paper airplanes were used to chart the progress of the contest.

J. A. Meiser, Jr.

848. A "four-way" sales contest. T. KNIGHT. *Milk Plant Monthly*, 38, 7: 40-41, 48. July, 1949.

In the "4-Leaf Clover" contest credits could be earned 4 ways. Selecting the first 28 d. in Jan. and dividing the sales for that period by two constituted the base period. The contests lasted for 7 consecutive 2-wk. periods. On regular milk sales retail routemen received 1 credit for every 28 qt. over the base period, whereas wholesale routemen had to sell 84 qt. over the base period to receive 1 credit. Homogenized milk credits were set up the same as for regular milk but tabulated separately to increase the sale of both products. An increase of 20 half-pints of coffee or whipping cream netted retail routemen 1 point; wholesale routemen needed 60 half-pints. Finally, an increase of 28 units of by-products netted 1 point for retail routemen; wholesale routemen needed 84 units for the same bonus. At the end of the contest period all sales in the 4 groups listed were totaled and the top routemen awarded prizes.

J. A. Meiser, Jr.

849. "Carry your carriers" to increase sale of by-products. P. L. ANDERS. *Milk Plant Monthly*, 38, 8: 28-29. Aug., 1949.

To offset the loss of regular milk sales during the summer, routemen transported certain by-products to the customers in their carriers. As an incentive to carry these by-products the sales manager visited certain customers on each routeman's route giving them an envelope containing a bonus voucher bearing a cash value of \$2.00 to \$10.00. Customers were instructed to give these envelopes to their routemen only if they came to the door and inquired about selling by-products from the carrier on their arm. For customers' participation free lb. of butter were given. Besides the bonus vouchers picked up by the men the plant offered an added \$100.00 prize to the person selling the most by-products during the 30-d. period.

J. A. Meiser, Jr.

850. Product costs. O. M. JOHNSON. *Can. Dairy Ice Cream J.*, 28, 5: 82-86. May, 1949.

The following reasons prove that product costs indicate whether the business is operating as it should: (1) All selling prices should be based on product costs, (2) Product costs point out the most profitable and least profitable products, (3) Correct valuation of inventories requires product costs, (4) Product costs point out where savings can be made, (5) Locating and stopping plant losses is facilitated through the use of product costs, and (6) Product costs indicate many of the methods necessary to conduct a business profitably. Total and unit product costs can be obtained only when costs and expenses are put together in the cost ledger with the plant reports.

The really pertinent question is not how much it costs to obtain product costs, but can one afford to be without them.

H. Pyenson

851. Wastes and losses in dairy plant operations. L. C. THOMSEN, Univ. of Wisc., Madison. *Milk Plant Monthly*, 38, 7: 26, 28, 30-32, 34. July, 1949.

A list of rules to be followed in the reduction or elimination of losses and wastes in dairy plants would be as follows: (a) obtain representative samples for analysis with the aid of proper sampling equipment and technique, (b) care for samples properly prior to analysis, (c) prepare and analyze samples properly, (d) select proper testing procedures to check the successive stages of your manufacturing operation, (e) install liquid level controls or signals to prevent overflows of milk, (f) check the accuracy of sanitary meters or volume gages that meter the amount of milk on hand, (g) install drip racks to collect drippings from cans, (h) repair leaky pipe lines, valves, etc., (i) check the final product for weight as well as composition, (j) determine the amount of milk waste passing into the waste disposal systems, (k) avoid the general use of water for rinsing milk wastes from the floor and (l) remember that wastes and losses in the plant are not confined to dairy products alone.

J. A. Meiser, Jr.

852. Improved bill collections. K. STRONG. *Milk Plant Monthly*, 38, 7: 62. July, 1949.

Rather than continually remind routemen that their collections are lagging, a board was erected that keeps the men posted. Every routeman who lowers his outstanding bills \$100 over the last base period receives a silver star. If a reduction of \$200 to \$300 is obtained a red star is given. So long as routemen keep getting stars, the company and the individual know that collections are improving.

J. A. Meiser, Jr.

853. Efficient fleet maintenance. R. MILLER. *Milk Plant Monthly*, 38, 7: 58-60. July, 1949.

The following procedures have done much toward simplifying and systematizing fleet maintenance in large plants: (a) routemen must take out and return their trucks to designated places in the garage, (b) all gas and oil placed in trucks must be recorded in triplicate and bear the routeman's signature as proof of delivery, (c) repair sheets are prepared by routeman and the work assigned to mechanics most capable of doing the necessary work, (d) individual record cards are kept for each truck bearing a complete record of operating and maintenance costs and (e) all trucks are given a monthly preventive maintenance inspection.

J. A. Meiser, Jr.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

854. Digestibility studies with ruminants. XIII. The effect of the plane of nutrition on the digestibility of linseed oil meal. C. J. WATSON, J. W. KENNEDY, W. M. DAVIDSON, C. H. ROBINSON and G. W. MUIR, Dept. of Agr., Ottawa, Canada. *Sci. Agr.*, 29, 6: 263-272. June, 1949.

Using 6 grade Shorthorn steers, digestibility trials were carried out on 6 rations, 1 of hay, predominately timothy, fed at the rate of 6.5 kg. and the others of 3 kg. of hay with linseed oil meal at rates varying from 1.0 to approximately 5.0 kg. Supplementary minerals and vitamins were given and the trial periods were arranged in a randomized Latin square set-up. The coefficients of digestibility in % were determined for dry matter, organic matter, nitrogen, ether extract, crude fibre, N-free extract and total carbohydrates and the means for the trials in which the mixed rations were fed were treated statistically.

The level of feeding had no influence on the digestibility of the nitrogen or on the ether extract. As the level increased, the digestibility of the carbohydrate fraction decreased. This decrease was statistically significant in the case of the total carbohydrates, dry matter and organic matter. The loss in TDN over the entire range of feeding was between 2 and 3%. O. R. Irvine

855. Digestibility studies with ruminants. XIV. The effect of the plane of nutrition on the digestibility of barley. C. J. WATSON, W. M. DAVIDSON, J. W. KENNEDY, C. H. ROBINSON and G. W. MUIR, Dept. of Agr., Ottawa, Canada. *Sci. Agr.*, 29, 8: 400-408, Aug., 1949.

This is a study duplicating the one described in the previous abstract, except that barley replaced linseed oil meal as the variable component of the ration. As the plane of nutrition increased the coefficients of digestibility decreased, significant differences being obtained in the case of all nutrients tested, i.e., dry matter, organic matter, nitrogen, ether extract, total carbohydrates and gross energy.

The plane of nutrition calculated in terms of maintenance requirements from Brody's formula indicated that the rations ranged from 0.75 to approximately 1.5 times that required for maintenance. O. R. Irvine

856. Composition and digestible energy of hays fed to cattle. T. G. PHILLIPS and M. E. LAUGHLIN. *J. Agr. Research*, 78, 10: 389-395. May 15, 1949.

The study was made on 25 samples of hay received from various laboratories in the U. S. These hays served as the sole ration in feeding experiments with cattle; of 18 samples tested there was a close relation between digestible and metabolizable energy. The lignin, protein, cellulose and crude fiber content of the samples all are related closely to their yield of energy but at different levels of these constituents for timothy and alfalfa. The lignin content serves as an excellent means of estimating the digestibility of energy and dry matter. Crude fiber content also is related significantly to the digestibility of energy, but less closely than the lignin content.

H. Pyenson

857. Carotene retention in alfalfa meal. Effect of moisture content. G. F. BAILEY, M. E. ATKINS, and E. M. BICKOFF. *Western Reg. Research Lab., Albany, Calif. Ind. Eng. Chem.*, 41, 9: 2033-6. Sept., 1949.

The influence of moisture levels of 0.5 to 26% on the rate of loss of carotene in alfalfa meal was determined under various conditions of storage. It is known that during storage where there is no access to air there is practically no loss of carotene. In alfalfa meal having access to air minimum loss of carotene occurred at a moisture level of about 8%, but only 70 and 25% are retained at 21 and 40° C., respectively, after 90 d. It is suggested that when the seal is broken on sealed samples the carotene is lost rapidly, increasingly so at higher moisture levels. Carotene in alfalfa meal cannot be preserved effectively by controlling the moisture content under conditions of free access to air at temp. above 20° C.

B. H. Webb

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

858. Fructose and citric acid assay in the secretions of the accessory glands of reproduction as indicator tests of male sex hormone activity. T. MANN, D. V. DAVIES and G. F. HUMPHREY, University of Cambridge, England. *J. Endocrinol.*, 6, 1: 75-85. Apr., 1949.

These studies were conducted with growing male rabbits, bull calves and young bulls. In the bull calves and young bulls, the seminal glands were weighed, examined histologically and analyzed for fructose and citric acid.

In bull calves 3-4 wk. of age, the seminal glands were poorly developed, the fructose content was below 8 mg./100 g. of seminal gland tissue and no citric acid was detected. In bulls 3-4 mo. of age, the seminal glands were enlarged,

there were some histological changes and the fructose values ranged from 42–108 mg./100 g. of tissue. In bulls 6–12 mo. of age, the tubules of the seminal glands were practically all canalized and the fructose content ranged from 95–475 mg./100 g. of tissue. In mature bulls, the seminal glands contained 420–870 mg. of fructose and 520–1120 mg. of citric acid /100 g. of tissue.

Six bull calves were castrated at 3 wk. of age. At the age of 8 mo., 2 of these calves were implanted with pellets of testosterone propionate and all 6 calves were slaughtered at 9 mo. of age. In the non-treated castrates, there was no citric acid in the seminal glands and little fructose. Although the fructose levels of the testosterone-treated castrate calves were not as high as controls of the same age, they did show a 6-fold increase in concentration over that of the non-treated castrates.

Spermatogenesis was first observed in calves 6 mo. old, although no mature spermatozoa were observed until animals were 12 mo. of age. Since the seminal glands function to secrete fructose and citric acid under the influence of testosterone propionate, and since they are both formed in the bull calf before the active appearance of spermatogenesis, the authors conclude that the testicular hormone in the bull begins to function before spermatogenesis. V. Hurst

859. The vaginal smear of the cow and causes of its variation. W. HANSEL, S. A. ASDELL and S. J. ROBERTS, Cornell Univ., Ithaca, N. Y. *Am. J. Vet. Research*, 10, 36: 221–228. July, 1949.

A careful study is reported of the changes in cell types found in the vaginal smear taken from normal cows at various stages in the estrus cycle and from cows treated with diethylstilbestrol and/or progesterone. Five distinct types of epithelial cells were tabulated at each examination and the presence of leucocytes and erythrocytes was noted. During estrus, few cornified cells were found and leucocytes were numerous. The percentage of cornified cells remained low until the eighth or ninth d. when it rose sharply and then remained high until 2 d. before estrus. Smears from ovariectomized heifers were so scanty that examination was unsatisfactory. Diethylstilbestrol-induced heat produced a cell picture very similar to that in a normal heat. Adding progesterone increased the percentage of cornified cells. E. W. Swanson

Also see abs. no. 860.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

860. Growth of first generation crossbred dairy calves. R. A. HILDER and M. H. FOHRMAN. *J. Agr. Research*, 78, 11: 457–469. June 1, 1949.

An analysis was made of the rate of growth of first generation crossbred dairy calves as compared with the growth standards for purebreds. The calves used represented crosses of Holstein, Jersey, Guernsey and Red Dane cattle. The measures of growth used are live weight and a set of 5 skeletal measurements. There is some indication that the crossbreds tend to be slightly larger than the mean of the parent breed averages. Heterosis is indicated in some groups by the fact that the crossbreds are significantly larger than the expected weight or measurement, but this does not hold true for all crossbred calves. Some interesting differences in breed interactions are shown, particularly in the Guernsey crosses.

H. Pynson

861. Live-stock watering device. A. H. HEMKER. (Assignor to General Electric Co.) U. S. Patent 2,479,355. 3 claims. Aug. 16, 1949. *Official Gaz. U. S. Pat. Office*, 625, 3: 787. 1949.

A bowl-shaped drinking fountain for cows, etc., has a treadle within the bowl, which on being depressed admits water to the bowl. The entering water and the water remaining in the bowl when the device is not in use are heated electrically to any controlled desired temperature.

R. Whitaker

862. The seasonal distribution of calf and milk sales in West Wales and the probable influence of climatic conditions on the rate of calving during the Autumn months and on the consequent milk production. R. PHILLIPS and J. L. DAVIES, University College of Wales, Aberystwyth. *J. Dairy Research*, 16, 1: 1–13. Jan., 1949.

Data are given which indicate that regional decreases in apparent winter conception rates are significantly similar to corresponding declines both in winter temperatures and in winter sunshine. E. L. Thomas

ICE CREAM

C. D. DAHLE, SECTION EDITOR

863. Flavor and texture major influence in ice cream buying. *Ice Cream Trade J.*, 45, 8: 48. Aug., 1949.

Flavor and texture are the 2 main factors which influenced consumers in their choice of brands of ice cream. Richness and smoothness were important to the majority. These and other facts about ice cream consumers' purchasing habits were revealed by a recent survey made in Lexington, Ky., by Merrill DeVoe of the University of Kentucky. Pints were the best sized sales

unit; 4:00 to 7:00 p.m. was the time when most ice cream is purchased for home use; and it is served more frequently for dessert than for a between-meal snack. Eighty-one % of the customers buy take-home ice cream regularly, with the drug store, ice cream and dairy store about equal as a source of supply, followed closely by grocery stores.

W. H. Martin

864. Stabilizers and emulsifiers, their use in the production of ice cream. R. B. REDFERN and W. S. ARBUCKLE, North Carolina State College. *Sou. Dairy Prod. J.*, 46, 3: 30-39. Sept., 1949.

Ice cream mixes containing (a) no stabilizer or emulsifier, (b) stabilizing products (gelatin, Dariloid, Permangel), (c) combination stabilizer and emulsifier products (Vestirine, Freeze-Text, Dricoid, Gelox, Duo-Lizer) and (d) stabilizer plus emulsifier (gelatin and Dariloid, each with EM 220, Special Na-Pe-Co, Mixacoid, Vis-Ca-Text, individually) were compared.

The products mentioned had no significant effect upon the pH of the mixes. All of the products increased viscosity. Stabilizer plus emulsifier increased viscosity least. The viscosity of mixes containing stabilizers increased most on aging with the exception of Dariloid mix which did not change viscosity. Stabilizers increased and emulsifiers decreased surface tension. The combination products produced no change and the stabilizers plus emulsifiers, with the exception of Dariloid plus Mixacoid, showed reductions in surface tension. Stabilizers increased the whipping time and emulsifiers decreased it. Stabilizer plus emulsifier decreased whipping time.

The use of emulsifiers resulted in smaller ice crystals and smaller air cells. The stabilizers and combination products increased the rate of melting and stabilizer plus emulsifier decreased melting rate. The combination products caused the ice cream to withstand heat shock best. All the products resulted in greater resistance to heat shock than the controls. Stabilizing and emulsifying agents tended to increase shrinkage, the greatest effect being produced by stabilizer plus emulsifier.

The effects of the use of stabilizers and emulsifiers were greater with batch freezing than with continuous freezing.

F. W. Bennett

865. Making good ices and sherberts. C. D. DAHLE. *Can. Dairy Ice Cream J.*, 28, 8: 27-29, 78-82. Aug., 1949.

Definitions of ices and sherbets, composition, sugars used, sugar and fruits, milk solids, stabilizers, acidity, overrun, freezing, flavoring and defects are discussed.

H. Pynson

866. Defects in ice cream and how to cure them. C. D. DAHLE. *Can. Dairy Ice Cream J.*, 28, 8: 62-66. Aug., 1949.

The defects reviewed are old material, unclean flavor, cooked flavor, neutralizer flavor, salty taste, sour taste, oxidized flavor, rancid flavor, sogginess, fluffy body, gummy body, crumbly body, coarse texture, buttery ice cream, sandiness, separation and curdiness, failure to melt and shrinkage.

H. Pynson

867. Creation of marbled patterns from semifluid substances. C. C. A. REETZ. (Assignor of one-half to L. A. Bloom.) U. S. Patent 2,479,261. 5 claims. Aug. 16, 1949. *Official Gaz. U. S. Pat. Office*, 625, 3: 763. 1949.

Ice creams having different flavors or colors are blended continuously in this device as the product leaves the freezer to produce a variegated or marbled effect. The blending is effected by bringing the ice creams together through tubes, one within the other, and mixing into the variegated condition by passage through spiral auger-shaped fins or blades within the single outlet tube.

R. Whitaker

868. Trends in ice cream packages. H. W. PICKELL. *Can. Dairy Ice Cream J.*, 28, 7: 31, 80. July, 1949.

An ice cream product that is easily purchased during shopping trips, an attractive package and a reasonably priced and good quality product are recommended to develop sales.

H. Pynson

Also see abs. no. 811, 830, 840, 841, 842.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

869. Significance of milk quality tests. W. H. CHILSON, Kansas State College Manhattan. *Milk Dealer*, 38, 10: 82-90. July, 1949.

The acidity test has little value other than a check on the very poorest quality milk. The natural acidity of milk varies from 0.13 to 0.20%; before a measurable acid rise of even 0.01% is evident the bacterial population must increase to at least 10,000,000/cc.

The odor test is much more reliable than the acidity test and as reliable or more reliable than taste. A well-trained man usually can pick out warm milk with a bacterial content of about 1,000,000/cc. and cold milk of 4 or 5 million/cc.

Neither the methylene blue nor the resazurin test is accurate enough for general use on high

quality milk. They are considered accurate on milk containing about 500,000 bacteria/cc or more.

The sediment test does not show much about the quality of milk. It may show clean milk production or just a good straining job on the farm.

The microscopic test is one of the most valuable in controlling the quality of a producer's milk. However, some technicians may over-rate their ability to look in the microscope and tell you just what is the trouble on the farm.

The standard plate count is the laboratory test usually used to determine the bacterial quality of low count raw milk and pasteurized milk. As a general rule it measures a given % of the total bacteria present, and as the number increases, either by contamination or growth, the plate count increases correspondingly.

A thermoduric count may enable the control department to detect unsanitary milking machines, pails, etc., that are not detected by other tests. The coliform test is of little value when used on raw milk. The test is used on pasteurized milk to determine contamination after pasteurization.

The phosphatase test is not a test of the bacterial quality of milk; it determines the presence or absence of the enzyme phosphatase and, therefore, is a test only for proper pasteurization.

The incubation test is a simple practical test to determine how long the milk or cream will keep at room temperature.

The selection of the tests that will be used for a particular milk supply will depend upon type of product sold, money spent for control, personal preference, laboratory facilities, amount of field work to be done and other factors. A test or tests should be selected that will best control the quality of the particular milk supply.

C. J. Babcock

870. How much is a quart? A. J. POWERS, Borden's Farm Products, Brooklyn, N. Y. Proc. 41st Ann. Convention Milk Industry Foundation, Plant Sec., vol. 2, p. 54. 1948.

Lack of uniformity exists between states in defining a quart measure of milk. Both the bottle fill-point and temp. of filling should be defined. Some states require filling to the cap seat, other to 0.25 in. below the cap seat, and several states have no fill-point requirement. In 10 states the vol. of liquid measure is specified at 68 to 70° F. When health department regulations concerning bottling temp. are adhered to (generally 50° F.), losses accrue to the milk co. due to the greater wt. of milk at the lower temp. than at 68° F. Milk shrinks 3.30 ml / qt. be-

tween 68 and 40° F. When 10,000 qt./d. are bottled at 40° F., an extra 75.24 lb. of milk are required to reach the fill-point established for 68° F.

D. J. Hankinson

871. Cream separator. L. C. COPEMAN. (Assignor to Copeman Laboratories Co.) U. S. Patent, 2,477,863. 4 claims. Aug. 2, 1949. Official Gaz. U. S. Pat. Office, 625, 1: 167. 1949.

Cream may be removed from a bottle of milk by this device consisting of a cylinder which just slides into the neck of the bottle. It is positioned by a flange located in about the middle of the cylinder, which fits snugly in the cap seat. The top outlet is restricted in size and is closed by the finger tip when device is removed from the bottle.

R. Whitaker

872. Concentrated sweet cream fat grinder. J. B. ORRELL. (Assignor to Abbotts Dairies, Inc.) U. S. Patent 2,479,080. 2 claims. Aug. 16, 1949. Official Gaz. U. S. Pat. Office, 625, 3: 717. 1949.

Blocks of frozen cream are pushed horizontally by a piston against a covered, rapidly rotating fan-like element which shaves the cream and discharges the comminuted material through an outlet below the whirling blades. The shaved cream is defrosted more readily than the original block of frozen cream.

R. Whitaker

873. Chocolate dairy drink. E. V. HAMMOND, J. H. MURRAY and W. A. WEIR. Can. Dairy Ice Cream J., 28, 5: 34-39. May, 1949.

Chocolate dairy drink increases the total consumption of fluid dairy products and, therefore, the total intake of Vit. A is greater. Chocolate dairy drink cannot be said, because of its 4 to 5% sugar content, to be a factor in dental caries. Cocoa taken in the form of dairy drink is not harmful and is recognized as a highly nutritious food.

H Pyenson

Also see abs. no. 806, 807, 808, 818, 819, 825, 826, 827, 831, 843, 844, 845, 846, 847, 848.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

874. Experiments on milking technique. 3. Combined effect of reducing the milking time and washing the udder with hot water. 4. Effect of increasing the milking time. F. H. DODD and A. S. FOOT, National Institute for Research in Dairying, Reading, England. J. Dairy Research, 16, 1: 14-22. Jan., 1949.

Twenty cows (17 Shorthorn and 3 Guernsey) all in declining lactation were divided into 5 blocks of 4 cows each. During the first 2 wk. the udders of all cows were washed with cold water (approximately 60° F.) about 1 min. before putting on the teat cups. During a 5-wk. experimental period the combined effect of reducing the milking time to 60% of the original flow period and washing the udder with hot water (approximately 120° F.) was studied. During a final 2-wk. period all cows were treated as in the initial control period. The data on milk yield indicate a complete lack of response to the change to washing the udder with hot water. Restriction of milking time to 60% of the original flow period caused an immediate abnormal decrease in yield with no recovery apparent throughout the experiment. A temporary increase in rate of flow resulted from the restriction in milk time; this was explained on the basis of increased udder pressure. The authors pointed out that none of the cows in the experiments was initially slow in letting down its milk and also that the experiments did not cover long-term training to a quicker flow.

In another experiment on 10 cows covering a 16-wk. period, neither the yield and quality of milk nor the rate of milking was affected by leaving the teat cups on at each milking twice as long as was necessary to carry out a normal milking.

E. L. Thomas

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

875. Reviews of the progress of dairy science. Section D. Nutritive value of milk and milk products. J. Dairy Research, 16, 1: 68-127. Jan., 1949.

This is a comprehensive review covering most of the literature relative to the above field published during the years 1942-1947 inclusive. 913 references.

E. L. Thomas

876. The effect on the biological value of bread nitrogen of additions of dried skim milk and of soya flour. K. M. HENRY and S. K. KON, National Institute for Research in Dairying, Reading, England. J. Dairy Research, 16, 1: 53-57. Jan., 1949.

Four lots of bread were baked using 85% extraction flour alone and others supplemented with 6% dry skim milk, 5.56% full-fat soya flour and 3% dry skim milk plus 2.78% soya flour, respectively. The biological values of the proteins

of the breads were determined on rats by the balance sheet method at an 8% level of protein intake. These were, respectively, 56.7, 59.3, 62.6 and 61.9 and the true digestibilities were 91.9, 90.6, 90.9 and 90.8. It was concluded that the soya-flour protein exerted a supplementary effect on the proteins of the plain and of the milk bread, whereas milk proteins showed no such supplementary effect, the effect being only additive; a possible explanation is that milk is known to be deficient in cystine, whereas heating renders the cystine of soya bean available to the animal organism, no deficiency of this amino acid existing in the heated product.

All except 1 of the samples of dry skim milk used possessed abnormally low biological values and hence were apparently deficient in lysine, as has been shown to be the case in deteriorated milk powders. The fact that the 1 sample of dry skim milk with a normal biological value of 81 failed to indicate a supplementary effect on bread protein is the only evidence against the possibility of a lysine deficiency being a factor in the experimental results reported.

E. L. Thomas

877. The influence of heat-processing on the functional and nutritive properties of protein. D. MELNICK and B. L. OSER. Food Tech., 3, 2: 57-71. 1949.

Casein, lactalbumin, skim milk, whole milk, dried skim milk, dried whole milk and other proteins were included in this study. It is proposed that the concept of food protein as $N \times 6.25$ should be extended to include a consideration of both the functional and nutritive properties of the protein. The functional properties affect appearance and palatability of the product and can be evaluated by determining the degree of protein denaturation. The nutritive properties of protein may be determined by animal assay but this data should be supplemented with *in vitro* tests. A procedure for determining the susceptibility of protein to enzymic digestion is given.

The more readily digestible food samples, according to the *in vitro* test, exhibited the higher biological value. Microbiologically available lysine was liberated from heated protein by pancreatin at a much slower rate than from the unheated product. For the optimal utilization of food proteins the essential amino acids all must be available for absorption and they also must be liberated during digestion at rates that permit mutual supplementation.

The formol titration curves of 14 amino acids are shown. A pH of 9.5 was selected as the end-point of the formol titration that gave the most reliable results. The nitrogen content of 18 foods

as determined by formol titration tended to average about 4% less than by the Kjeldahl analysis.
E. R. Garrison

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

878. Goitrogenic effect of aminothiazole and reactions of thymus and lymphoid tissue. C. GREGOIRE, Fondation medicale Reine Elizabeth and Fonds National de la Recherche Scientifique, Brussels, Belgium. *J. Endocrinol.*, 6, 1: 14-22. Apr., 1949.

Male and female white rats ranging in age from 6-8 wk. were used. Animals studied were controls, 2-aminothiazole fed, and those which were incompletely thyroidectomized, incompletely thyroidectomized and given 2-aminothiazole, thyroidectomized, and thyroidectomized plus 2-aminothiazole. The 2-aminothiazole was administered in the drinking water as a 0.1% solution for 21 d. Measurements included the wt. and histology of the thyroids, thymus, spleen and lymph nodes, the wt. of the adrenals, the histology of the kidneys and body growth.

Growth was retarded in all groups except in those rats that were incompletely thyroidectomized. In animals given aminothiazole, the thyroid glands weighed more than 3 times those of the controls on an av. relative wt. basis. Histologically, aminothiazole produced thyroids with high columnar epithelium and devoid of colloid. Thyroidectomy or aminothiazole administration either impaired the growth of the thymus or caused involution to the extent that the thymus glands of these animals weighed only one-half as much as the thymus glands of the controls. Histologically, neither thyroidectomy nor aminothiazole administration caused any striking changes in the splenic tissues. Aminothiazole administration superimposed upon thyroidectomy did not cause a greater change in thymus weights or histology than did thyroidectomy alone. In the dosages administered, no significant changes in wt. or histology of the other glands studied were brought about by aminothiazole administration.

It is suggested that the action of aminothiazole on the thymus is not direct but that it is mediated through the thyroid gland.
V. Hurst

879. The Sulkowitch test as a guide in the diagnosis and therapy of bovine hypocalcemia. D. K. DETWEILER and J. E. MARTIN. Univ. of Penn., Philadelphia. *Am. J. Vet. Research*, 10, 36: 201-207. ^g 1949.

The Sulkowitch test for urine Ca has been proposed as a means of differentiating hypocalcemic conditions from others which give similar symptoms. The test is a precipitation of Ca as oxalate at pH 2.6 to 4.5. Cows with hypocalcemia (below 8.4 mg. % serum Ca) usually gave a negative test. Some cases of normal blood Ca were observed with negative Sulkowitch test. Hypercalcemia was always accompanied by a heavy positive Sulkowitch test. Variables such as urine pH, concentration, blood Ca level and manipulation of the test affect the agreement between serum Ca and urine Ca. As a clinical test, it should be of value in preventing Ca injections for animals already hypercalcemic and confirming suspected cases of hypocalcemia before treatment.

E. W. Swanson

880. Renal excretion following intravenous injection of calcium salts in the normal cow. A. H. CRAIGE, JR., R. B. JOHNSON, E. G. BLACKBURN and J. M. COFFIN, Univ. of Md., College Park. *Am. J. Vet. Research*, 10, 36: 217-220. July, 1949.

Intravenous injections of 10.5 g. Ca in 500 cc. were given as Ca borogluconate and CaCl_2 to each of 4 normal cows, one lactating. Urine was collected continuously for 1 to 2 hr. before injection and 2 or 3 hr. afterward. Blood samples were taken before injection and 3 min., 1, 2 and 24 hr. post injection. Analyses were made of blood for Ca and P and of urine for rate of excretion, pH, carbonates, NH_3 , Ca, P, Cl, organic acids, creatine, creatinine, volatile phenols, hydroxyacids and residual phenols in order to correlate changes with recovery rate of cows treated for milk fever. Although both Ca compounds are effective in milk fever treatment, their effects on renal excretion were markedly different. A rise in blood and urine Ca and a decrease in urinary output of volatile phenols was common to both treatments. Ca gluconate caused an increased urine volume while CaCl_2 produced a decrease. Diarrhea followed injections of both compounds. No consistent change in P was noted and P was found in urine only in traces. E. W. Swanson

881. Process for producing synthetic thyroprotein. C. W. TURNER and E. P. REINEKE. (Assignors to American Dairies, Inc. and the Quaker Oats Co.) U. S. Patent 2,478,065. 8 claims. Aug. 2, 1949. Official Gaz. U. S. Pat. Office, 625, 1: 219. 1949.

A protein, such as casein, is iodinated at a temperature of 15-70° C and at a pH of 6.8-10.0 until the Millon test is negative. The iodinated protein is then heated for 12-72 hr. at 50-100° C with MnO as a catalyst.
R. Whitaker

882. The fractionation of γ -globulin by electrophoresis-convection. J. R. CANN, R. A. BROWN and J. G. KIRKWOOD. Calif. Institute of Technol. J. Am. Chem. Soc., 71, 8: 2687-2691. Aug., 1949.

Because of its known heterogeneity and immunological importance, sub-fractionation of γ -globulin, Fraction II of bovine plasma obtained by ethanol fractionation, was attempted by application of the method of electrophoresis-convection. Employing the isoelectric procedure, whereby the protein components successively are immobilized at their respective isoelectric points, γ -globulin was separated into 4 fractions of different mean mobilities and isoelectric points.

Fraction A, representing 45% of the original γ -globulin, corresponds roughly to the bovine γ_2 -globulin of Hess and Deutsch. It had a mobility of -1.35×10^{-5} and an isoelectric point of 7.03, 0.5 pII Unit greater than the mean isoelectric point of γ -globulin. Fraction B appears to correspond to the bovine γ_1 -globulin of Hess and Deutsch, which has a mobility of -2.1×10^{-5} .

The second stage of fractionation of bovine γ -globulin resulted in a top fraction, Fraction C, having a mobility of -1.63×10^{-5} and an isoelectric point of 6.47. Fraction D, the bottom fraction, had a mobility of -2.20×10^{-5} and an isoelectric point of 6.01, which is about 0.5 pII unit lower than the mean isoelectric point of γ -globulin. Fraction C represented 19% and Fraction D 36% of the original γ -globulin.

When properly normalized and combined, the gaussian mobility distributions of these fractions yield a mobility distribution in agreement with that of γ -globulin itself. H. J. Peppler

Also see abs. no. 858.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

883. Easier and more effective cleaning methods. JOHN R. PERRY, Sealtest, Inc., New York. Proc. 41st Ann. Convention Milk Industry Foundation, Plant Sec., vol. 2, p. 32. 1948.

Cleaning methods in use in many dairies are described as primitive. The lack of available equipment has prompted the designing of certain appropriate pieces of equipment of importance in efficient cleaning.

Hot water from an electric hot water generator of the reservoir type is mixed with cold water by means of a tempering valve. This water at 115° F. is supplied to all hose stations, each of which is equipped with a pressure regulating valve for supplying no more than the necessary pressure to

perform the cleaning. A special lightweight 0.5 in. creamery hose is provided at the hose stations; this is more economical and at the same time delivers an adequate amount of water. Each hose is equipped with a rubber-covered shut-off valve at the discharge end. The operator can control the water flow without making trips to the hose stations.

Specially designed tanks for cleaning separator and clarifier parts and sanitary fittings make use of rotating brushes to which cleaning solution is fed. Rinsing is accomplished in a separate compartment by means of a foot-controlled spray. A sanitary fitting "buggy" is provided for transporting parts to be washed to the wash sinks. Solution-fed brushes, equipped with long-wearing nylon bristles, are provided for cleaning larger stationary equipment such as coolers, vats, etc. Some types of solution-fed brushes are equipped with a rotating brush, powered by an air turbine.

Improvements in spray equipment for applying chemical sterilizing agents also have been made.

Economics result from decreased labor for cleaning, better cleaning, reduced fuel consumption, lower water usage, lower hose costs, decreased brush expenditures and reduced usage of cleaning compounds. D. J. Hankinson

884. Acid cleaners—their value and proper use. C. B. SHOGREN, Klenszade Products, Inc., Beloit, Wis. Proc. 41st Ann. Convention Milk Industry Foundation, Plant Sec., vol. 2, p. 40. 1948.

The conventional alkaline cleaners used for many years possessed certain limitations, chief of which were poor emulsification, peptizing action and rinsing. Hard water likewise imposed a problem. The development of suitable organic acids together with the new wetting agents made possible a cleaning combination which overcame the limitations of the alkaline cleaners. This combination, referred to as an acid cleaner, but more properly called an organic cleaner, possesses one shortcoming in that fat is not effectively removed. Continued use leads to a dark greasy film on equipment.

An effective cleaning program can be followed to take advantage of the beneficial effects of both alkaline and acid cleaners by using both materials but at different times. Under normal conditions an alkaline cleaner is used for 3 days and on the 4th day the acid cleaner is used. With very hard water the acid cleaner should be used every other day. Milk stone is kept under control without resorting to arduous scrubbing to remove built-up deposits. D. J. Hankinson

885. Combined cleaner-sanitizing agents—their advantages and limitations. G. J. HUCKER, New

York Agr. Expt. Sta., Geneva. Proc. 41st Ann. Convention Milk Industry Foundation, Plant Sec., vol. 2, p. 44. 1948.

A single compound was sought which would accomplish both cleaning and sterilizing in 1 operation and which also would be effective in cold water. Alkaline cleaners were unsatisfactory because they were not compatible with available sterilizing agents. The wetting agent or anionic type of cleaner was not considered satisfactory because cleaning was not effective and also because the germicidal action of the sterilizing component of the mixture was reduced. The non-ionic group of cleaners was most favorable because of compatibility with sterilizing agents, particularly the quaternary ammonium compounds. However, the non-ionic compounds were not sufficiently effective as cleaners without some modification. The addition of the "proper alkali" is recommended to enhance the cleaning qualities.

Studies with modified non-ionic quaternary mixtures for cleaning milking machines and for use in dairy and food plants indicated very satisfactory performance. Marked reductions in raw counts and thermoduric counts were observed. Washing by flushing with the solution was not effective and led to a slimy condition on certain milking machines. Brushing of rubber parts was recommended. A dispenser which feeds into cold water lines is suggested for dairy plant use to aid cleaning operations.

D. J. Hankinson

886. Corrosion by commercial sodium hypochlorites and its inhibition. G. H. BOTHAM and G. A. DUMMETT, A.P.V. Laboratories, London, England. J. Dairy Research, 16, 1: 23-38. Jan., 1949.

Nine samples of commercial hypochlorites were corrosive at 150 p.p.m. available chlorine and 40° C. to metals such as aluminum, tinned copper, nickel silver and cast stainless steel (18 Cr, 8 Cu, 3 Mo). Hypochlorites containing KMnO_4 when aged were found to attack wrought 18/8 stainless steel.

Sodium silicate added at the rate of 0.25% by vol. to hypochlorites diluted to 150 p.p.m. available chlorine effectively inhibited corrosion of all metals studied, with the exception of aluminum which showed slight attack after 24 hr. contact. The silicate ion apparently exerts a specific effect, since additions of NaOH and Na_2CO_3 to the same pH were not effective and actually increased attack on aluminum. Increase of pH from 9 to 10.5 by the addition of either Na_2CO_3 or sodium silicate resulted in an equal and significant reduction of bactericidal efficiency of the hypochlorites.

E. L. Thomas

887. Field testing for quaternary ammonium compounds. W. K. MOSELY. W. K. Mosely Laboratories, Indianapolis, Ind. Milk Plant Monthly, 38, 7: 76-77. July, 1949.

The object of this investigation was to perfect a rapid, sensitive, yet simple method for determining quantitatively quaternary ammonium compounds in concentrations of 10 to 300 ppm. The resulting test is conducted as follows: to 1 ml. of the solution to be tested is added 0.1 ml. of a 5% citric acid buffer and 1 ml. of standard eosin indicator and the contents shaken until a pink color appears in the lower layer. The contents of the test tube then are titrated with a 0.01% di-octyl sodium sulfosuccinate solution until the red color disappears or becomes white; the ppm. of quaternary compound may then be read from a standard reference curve.

This method was proven accurate for 3 commercial quaternary germicides. Also it could account for all the quaternary compounds added to a 1% skim milk solution. All tests must be as near pH 3.5 as possible if an accurate determination is to be had. A modification of the above method involving paper strips saturated with eosin solution also was perfected and could be used as a rough estimate of the quaternary present.

J. A. Meiser, Jr.

Also see abs. no. 816, 824.

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

BOOK REVIEW

888. Methods of vitamin assay. Assoc. of Vitamin Chemists, Inc., Interscience Publishers, Inc., N. Y. 189 pp. 1947.

This volume is based upon actual laboratory check of methods for Vitamin A, carotene, thiamin, riboflavin, niacin and ascorbic acid. Chemical formulae, spectroscopic characteristics (except for niacin and ascorbic acid), sources, relative precision of methods and detailed methods of assay are presented for the vitamins listed above. Literature references are presented for assay of vitamins, D, E and K and for biotin, folic acid, *p*-amino benzoic acid, inositol, choline, pantothenic acid and pyridoxine. An excellent section is presented relative to sampling, though unfortunately, dairy products are slighted in this section. The volume is written critically and should be in the libraries of all who are engaged in vitamin work.

E. W. Bird

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

889. Incidence of Q fever in eastern Washington. R. DODDANANJAYYA, State College of Wash., Pullman. Pub. Health Repts., 64, 39: 1230-1236. Sept. 30, 1949.

A seriological survey showed that Q fever exists in eastern Washington in both humans and animals. Six of 289 samples of human sera were found to have Q fever complemented-fixing antibodies, in titers ranging from 1:8 to 1:128. Three of these positive sera were from humans having close contact with animals while the other 3 persons had no occupational contact with animals. Out of 327 samples of blood sera from beef and dairy cattle, 9 were found to be positive with titers ranging as high as 1:128. The breed, age and sex of the animals had no special significance.

D. D. Deane

BUTTER

O. F. HUNZIKER, SECTION EDITOR

890. Smörets struktur vid vanlig och kontinuerlig smörtillverkning. (The structure of butter by the usual buttermaking process as compared with the continuous method.) N. KING. Svenska Mejeritidningen, 40, 9: 95-97. Feb., 1948. 40, 10: 105-109. March, 1948.

The effect of physical and chemical factors on the structure and consistency of butter is discussed in detail. The microscopic structure of butter consists of fat globules, fat crystals, brine droplets and air cells. Liquid butter fat serves as embedding medium for these elements. Fe and Cu present in cream in combination with phospholipids and proteins are believed to be transferred to the butter. The number of fat crystals in free fat and their size are believed to play an important part in the hardness and consistency of butter. The size and number of brine droplets may have some influence upon the appearance and keeping quality of butter.

In the phase inversion of the cream as it occurs in the Alfa process for continuous butter-making the following steps are enumerated: (a) fat globules distributed in milk serum, (b) beginning clumping with fat globules partly combined in clumps, (c) very large clumps with an irregular edge, (d) double emulsions in which both brine droplets, containing larger or smaller numbers of fat globules are present in the fat phase and fat globules with a birefringent peripheral layer and (e) a system with fat globules and brine droplets in free fat and no fat globules in brine droplets.

It is pointed out that microscopic methods are of considerable value in research involving butter structure but the microscopic method is useful only when dimensions are not smaller than 0.5-0.1 μ . Undoubtedly there are present in butter, particles varying from 0.1 μ to 1m μ such as small fat crystals in free fat, water veins and phospholi-

pid-protein membranes of fat globules. In order to obtain more information regarding these, it will be necessary to use methods that will be as satisfactory in the submicroscopic field as the microscopic methods are in the microscopic field.
G. H. Wilster

891. Metallic churns and butter kneaders having no kneading rollers. F. J. J. HENRARD. (Assignor to Ecremeuses Melotte.) U. S. Patent 2,481,842. 2 claims. Sept. 13, 1949. Official Gaz. U. S. Pat. Office, 626, 2: 497. 1949.

This metallic churn and butter kneader rotates on its horizontal axis, the 2 end walls being flat and parallel. Instead of the usual cylindrical shape, with the cross-section in the shape of circle, the cross-section of this churn is in the shape of 3 equal cyloidal curves, thus providing suitable agitation for churning and working action for the butter as the churn is rotated.

R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

892. Cheese cutting machine. L. M. SEELY. U. S. Patent 2,481,162. 3 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, 626, 1: 203. 1949.

Cheese may be sliced in any shape by this vertical gravity-operated knife. A guard prevents operator injury by the knife.

R. Whitaker

893. Rotary drum cheese grater. J. ORLANDO. U. S. Patent 2,481,336. 1 claim. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, 626, 1: 248. 1949.

A rotating drum having a rough surface grates the cheese as it is fed continuously to the drum.

R. Whitaker

894. Manufacture of rennet paste. E. C. SCOTT and G. W. McDONALD. (Assignors to Swift and Co.) U. S. Patent 2,482,520. 4 claims. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, 626, 3: 809. 1949.

A paste is made of 60 parts whole milk curd and 1 to 12 parts of rennet extract and the pH adjusted to 4 to 5.

R. Whitaker

Also see abs. no. 897.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

895. Mechanism of the fermentation of lactose by yeasts. MORRISON ROGOSA, U. S. Dept. of

Agr., Washington, D. C. J. Biol. Chem., 175, 1: 413-423. Aug., 1948.

Evidence is given that enzymatic hydrolysis of lactose to the component monosaccharides is not necessary for fermentation. Ten different lactose-fermenting organisms were used in the study.

A. O. Call

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

896. Methods for determining the iron content of milk. F. A. JOHNSTON, NAOMI GELLMAN and JUNIATA STROM, Cornell Univ., Ithaca, N. Y. J. Biol. Chem., 175, 1: 343-347. Aug., 1948.

In 1943 the National Research Council reported the Fe content of milk as 2.0 mg./kg. This figure was lowered to 0.7 mg./kg. in their 1945 published tables. In 1944 the senior author reported a value of 0.32 mg. Fe/kg. A recheck of the dry ash method originally used showed it to be reliable when compared with two other methods. Ten separate milk samples from 5 distributors (presumably from the Ithaca area) were tested for Fe by 3 methods. The average values by each method were 0.38, 0.39 and 0.33 mg./kg. The highest reported value of any sample by any method was 0.59, and the lowest 0.25 mg./kg. Details of an improved wet ashing procedure are given.

A. O. Call

897. The estimation of fatty acids of intermediate chain length by partition chromatography. M. H. PETERSON and M. J. JOHNSON, Univ. of Wis., Madison. J. Biol. Chem., 174, 3: 775-789. July, 1948.

While investigating the role of fatty acids in Cheddar cheese flavor a chromatographic method for the quantitative estimation of formic, acetic, propionic, n-butyric, caproic, caprylic and capric acids was developed. A detailed description for the preparation of both macro and micro chromatogram tubes and their development, as well as the titration of aliquots of the effluents is given. Sulfuric acid (27 to 35 N) was used as the non-mobile phase and Celite 545 as an inert filler. For routine estimations in biological materials one macro and three micro columns were used. Analyses of known mixtures, as well as butterfat with added known amounts of fatty acids showed recoveries with an 8% maximum error.

A. O. Call

898. Stable fortified milk products and process of preparing same. G. E. GRINDROD. (Assignor

to Wis. Alumni Research Foundation) U. S. Patent 2,481,414. 11 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, 626, 1: 268. 1949.

An insoluble salt of Cu, Fe or Mn is allowed to absorb an edible protective colloid and the dispersion is incorporated into liquid milk products to form a fortified concentrated sterilized food.

R. Whitaker

899. Stable milk product containing added anti-anemia factor and process of making same. G. E. GRINDROD. (Assignor to Wis. Alumni Research Foundation.) U. S. Patent 2,481,415. 7 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, 626, 1: 268. 1949.

Essentially the same as Abstract 898, except that ascorbic acid is added to the product before canning and sterilizing.

R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

900. Practical ammonia refrigeration. Cooling buttermilk, condensed milk and ice cream mix. C. H. MINSTER, Greenbriar Dairy Products Co., Beckley, W. Va. Ice Cream Rev., 33, 2: 52, 54, 56, 57, 58, 60. Sept., 1949.

The refrigeration load as applied to cooling buttermilk involves cooling the fluid skim milk from the pasteurization temperature of 180-190° F. to the setting temperature of 70-74° F., and cooling of the finished product from the setting temperature down to 50° F. or below. Surface coolers, plate-type coolers or coil vats may be used in cooling the fluid skim milk. Vat cooling with sweet water or brine is the most common method employed in cooling buttermilk from the setting temperature to 50° F. or below. Freezing of any part of the buttermilk and excessive agitation during cooling are to be avoided.

Sweetened condensed milk may be cooled in a coil vat or by an internal tubular cooler. The latter provides for continuous cooling from the pan with no intermediate crystallization period required. The well water temperature and the final temperature desired are variables which will affect materially the refrigeration load in cooling sweetened condensed milk.

Plain condensed milk may be cooled with a vat or plate-type cooler. The latter method appears to be growing more popular. Precautions should be taken to avoid contamination of the product and to prevent the incorporation of air during

the cooling process. Evaporated milk, unless it is to be stored over night, need not be cooled prior to canning and sterilization.

Ice cream mix, because of its greater viscosity, which interferes with heat transfer, is more difficult to cool than fluid milk. To cool ice cream mix efficiently, either the surface area of the cooler must be increased or the time allowed for cooling must be increased. Regeneration has only limited application in cooling ice cream mix, since the sugar and stabilizer must be dissolved in the mix prior to pasteurization.

Data presented show that if an ice bank storage system is used, the hourly refrigeration load may be reduced by as much as 90% as compared with the use of direct expansion. W. J. Caulfield

901. Milk cooler. G. R. DUNCAN. U. S. Patent 2,482,579. 6 claims. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, 626, 3: 826. 1949.

This tank for cooling milk in cans is characterized by having a side door for easy insertion of the cans. A cooling coil below the can-supporting frame provides chilled water which is circulated and sprayed over the cans in the tank.

R. Whitaker

902. Ice cream package filling mechanism. F. D. PALMER. U. S. Patent 2,482,593. 1 claim. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, 626, 3: 829. 1949

A sleeve slides in and out of the end of the tube leading from a continuous freezer. When the sleeve is projected, the valve on the end of the sleeve is closed and it is open when retracted, thus alternately filling and discharging a given portion of ice cream as the sleeve is moved up and down.

R. Whitaker

903. Method of and apparatus for dehydrating liquid products. J. M. HALL. (Assignor to Drying and Concentrating Co.) U. S. Patent 2,481,418. 14 claims. Sept. 6, 1949. Official Gaz. U. S. Pat. Office, 626, 1: 269. 1949.

Milk is heated rapidly under pressure to temperatures sufficient to destroy instantly bacteria and enzymes and immediately sprayed into a blast of air at a lower pressure to reduce the moisture content of the milk.

R. Whitaker

904. Centrifugal separator with movably supported supply can therefor. W. H. HARSTICK and O. E. HEINTZ. (Assignors to International Harvester Co.) U. S. Patent 2,482,272. 6 claims.

Sept. 20, 1949. Official Gaz. U. S. Pat. Office, 626, 3: 745. 1949.

The supply tank which feeds this separator may be raised and lowered by means of a foot pedal at the base of the machine. When lowered, the supply tank outlet is positioned correctly for feeding directly into the spinning bowl.

R. Whitaker

905. Centrifugal homogenizer. H. BECHIA. U. S. Patent 2,482,235. 2 claims. Sept. 20, 1949. Official Gaz. U. S. Pat. Office, 626, 3: 735. 1949.

The fluid to be homogenized is introduced through a pipe into a rapidly rotating horizontal drum from which it is discharged rapidly by centrifugal force through small perforations in the outer wall of the drum, to impinge on serrations mounted close to the rotating drum. The homogenized product drains into a bowl-like vessel below the spinning drum and is discharged through an outlet in bottom.

R. Whitaker

906. Dump can. V. SCHWARZKOPF. (Assignor to Lathrop Paulson Co.) U. S. Patent 2,480,778. 3 claims. Aug. 30, 1949. Official Gaz. U. S. Pat. Office, 625, 5: 1415. 1949.

The features of this easily cleaned dump tank for milk are a simple means of preventing splashing when cans of milk are rapidly emptied into it and the inclusion of a removable strainer tray.

R. Whitaker

907. Building heating. J. C. McCABE, McGraw-Hill, New York, N. Y. Operating Engineer, 2, 9: 19-34. Sept., 1949.

Topics covered in this review are, behavior of heat, calculation of heat loads, infiltration, distribution of heat, distribution systems, heating units and auxiliaries. There are 81 tables and illustrations. Some of the heat distributors illustrated and discussed are radiators, wall radiators, convectors, baseboard heaters, panel heaters, blast coils, unit ventilators and propellor-fan units. The discussion of auxiliaries concerns valves, fittings and traps.

H. L. Mitten, Jr.

Also see abs. no. 891.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

908. Keep out of the hole with cost control. F. MERISH. Milk Plant Monthly, 38, 9: 34-36. Sept., 1949.

The 14 rules discussed for maintaining an effective cost control in the dairy plant are: (a) accurate accounting systems, (b) monthly profit and loss statements, (c) analysis of business figures, (d) comparative analysis by percentages, (e) depreciation in cost allotments, (f) overhead expense, (g) promotional outlay increases, (h) equipment modernization, (i) provisions for personal salary, (j) production cost knowledge, (k) departmentization of cost and sale figures, (l) insurance costs, (m) delinquent accounts and (n) improved supervision.

J. A. Meiser, Jr.

909. Separate cabinets for "Take Home" sales build volume at drug stores. V. M. RABUFFO. Ice Cream Trade J., 45, 9: 28, 31, 65-69. Sept., 1949.

The installation of separate serve-yourself cabinets for dispensing packaged ice cream may save drug stores their "take home" ice cream market. This would simplify proper pricing, eliminate waiting on rushed fountain clerks and, with carry-home provisions and proper location in the store, would increase sales and retain ice cream customers who rapidly are turning from drug stores to other retail outlets.

W. H. Martin

910. Bonus plan spurs sales. P. L. ANDERS. Milk Plant Monthly, 38, 9: 68-69. Sept., 1949.

Using the previous month's sales as the base period, increased sales of 1 to 3 points netted the routeman \$1.00 per point. Four to five points obtained \$1.50 each, whereas 6 points returned \$2.00 per point. Besides being used for milk sales, this plan was used also for increasing buttermilk and cheese sales.

J. A. Maiser, Jr.

911. Collecting "Slow accounts". I. FANALD. Milk Plant Monthly, 38, 9: 38-39. Sept., 1949.

A drastic reduction in slow accounts was accomplished by the issuing of "nudge cards" to routemen whose duty was to attempt a collection each time they found a delinquent customer home. If, at the month's end, payments had not been made, red stickers were pasted on the face of the bill demanding immediate payment and these in turn delivered by the routeman to the customer.

J. A. Meiser, Jr.

912. What it costs to serve dealers' customers of different sizes. P. P. MILLER, General Ice Cream Corp., Schenectady, N. Y. Ice Cream Trade J., 45, 10: 60. Oct., 1949.

The cost per gallon to serve customers of one of General Ice Cream Co.'s plants ranged from \$0.2757 for customers of the 6001 to 7000 gal. group to \$0.9035 for the 101 to 200 gal. group. These figures include delivery, selling and administrative expenses. To make the same profit per gallon on large and small accounts, the price to the small account may have to be very high.

W. H. Martin

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

913. Voederproeven over de invloed van organische zuren (vooral in verband met het aanzuren van ondermelk). (Feeding trials on the influence of organic acids, especially in relation to the acidifying of skimmilk.) (English summary.) TH. J. DEMAN, Institute for Modern Cattlefeeding, "De Schothorst", Hoogland near Amersfoort, Holland. Publication of the Nationale Coöperative Aan-en Verkoopvereniging voor de Landbouw (National Cooperative Buying and Selling Association for Agriculture.) "Centraal Bureau" G. A., Rotterdam, Holland. 31 pp. 1949.

Skimmilk, used in practice for fattening pigs and raising calves, often gave bad results caused by putrefying bacteria present in skimmilk of inferior quality. This can be improved by using the ripened product. Experiments were performed in fattening pigs with skimmilk acidified with lactic acid, acetic acid, formic acid and citric acid to a pH of about 5.7, and compared with culture-ripened product and sweet skimmilk. The artificially acidified skimmilk caused approximately the same rate of growth and food consumption as the culture-ripened product. Best results were obtained with the sweet skimmilk which was of good quality. With citric acid a somewhat retarded growth and a disturbance in the locomotion of the animals were observed. For practice, formic acid is most efficient, being cheap and having a low equivalent weight.

A growing experiment with calves, comparing skimmilk acidified with formic acid and culture-ripened skimmilk showed the formic acid product as favorable as the other one. An experiment on the influence of addition of 0.1% of citric acid to the fattening mash of pigs resulted, at times, in disturbances in the locomotion and in other cases in cannibalism (biting off each other's tails). Possibly citric acid works in this way via disturbance of the microflora in the intestines, influ-

encing the production of B vitamins. The observed abnormalities, curable with yeast, may be caused by shortage of riboflavin and pantothenic acid, but this explanation needs further investigation.

A. F. Tamsma

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

914. Inheritance of a Karakul-type curliness in the hair of Ayrshire cattle. F. E. ELDRIDGE, F. W. ATKESON and H. L. IBSEN, Kansas State College. J. Heredity, 40, 8: 205-214. Aug., 1949.

The Karakul-type of curliness involving irregularity in the diameter of the hair rather than uniform flatness was found in an Ayrshire herd. The curliness is pronounced at birth, resembling a newborn Karakul lamb, and becomes less curly with age. The seasonal variation noted was concluded to be caused by the shorter hair in summer when the curliness is less evident, as compared to winter when the curliness is characteristic. There is no sex difference in the expression of the responsible gene(s). The character was concluded to be due to a single autosomal dominant gene, *K*, and differs from the more common, variable type of curliness found in individuals of most breeds, and from the type of curliness associated with semi-hairlessness. L. O. Gilmore

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

915. Field tests of insecticides and spraying methods to control horn flies in dairy herds. W. S. MCGREGOR, U.S.D.A., Agr. Research Adm., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 4: 641-643. Aug., 1949.

DDT, DDD, methoxychlor and toxaphene emulsions, each containing 0.5% of the toxicant, were compared for horn fly control in field tests on Jersey cattle in Texas. Spray application was made on cattle confined in stanchions. A nozzle pressure of about 200 lb./in.² was used. Treatments began in June, when fly population averaged 25 or more per animal in every herd, and were repeated each time the population rebuilt to 25 per animal. Area of cattle treated and quantity of spray varied as follows: (a) 2 qt. on entire body, (b) 1 qt. on entire body, (c) 1 qt. on top line and (d) 1 qt. on underline.

There was great variation in range of pro-

tection periods against flies as afforded by insecticides. One qt. of any spray applied on the top line was as good as 1 or 2 qt. on the entire body. Underline treatment was less effective, except with toxaphene. E. H. Fisher

916. The DDT content of milk from a cow sprayed with DDT. R. H. CARTER and H. D. MANN, U.S.D.A., Agr. Research Adm., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 4: 708. Aug., 1949.

A high pressure sprayer was used to apply a wettable powder suspension at a concentration of 4 lb. of DDT/100 gal. of water. A Shorthorn herd was sprayed over the entire body to the point of super saturation. Milk samples were taken from 1 cow for DDT analysis. Milking was by hand, and no precautions to prevent contamination of milk with DDT were taken. No check sample of milk was taken before spraying. Beginning 2 d. after spraying, and at 5 subsequent irregular intervals, milk samples showed a range of from 3.0 to 0.4 p.p.m. of DDT over a period of about 5 wk.; the average was 1.3 p.p.m.

E. H. Fisher

917. Cows heat house. N. HOLMQUIST, Swedish Government Research Institute for Farm Buildings, Lund, Sweden. Agr. Eng., 30, 9: 425. Sept., 1949.

An average milking cow produces about 20,000 cal./d., 25% of which is latent in respiratory moisture. All of the latent and 60% of the sensible heat is wasted by being carried away by ventilating currents without heating the barn. The remaining heat is sufficient for keeping the temperature high enough.

The Swedish project attempts to use the waste heat from the barn for heating a 5-room house. A heat pump with its evaporator located in the barn's outgoing ventilating duct is used. The lowest design temp. for which the system is calculated is 5° F. The system with 10 to 15 milking cows will heat the house with no insulation on the house or barn. When heating oil is \$45 per ton and electricity is 2¢/kw.-hr., the cost of heating is \$245 with oil and \$190 with the heat pump. The system was tried in Sweden last winter and found to be practical.

H. L. Mitten, Jr.

918. Portable milker. A. I. TUPENING. (Assignor to DeLaval Separator Co.) U.S. Patent

2,482,602. 5 claims. Sept. 20, 1949. Official Gaz. U.S. Pat. Office, 626, 3: 831. 1949.

A milking machine, complete with vacuum pump, is mounted on a 2-wheeled cart.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

919. Use of whey in sherbets. F. E. POTTER and D. H. WILLIAMS, Agricultural Research Administration, USDA. Ice Cream Trade J., 45, 9: 54-55, 86-88. Sept., 1949.

Approximately 75% of the 12 billion pounds of cheese whey produced annually is used as animal feed or wasted. Ransdell and Webb developed a sweetened condensed whey to overcome the high water content and perishable nature.

A good quality sherbet was made by using whey solids instead of milk solids. These sherbets contained 5% whey solids when made from plain condensed, sweetened condensed, or dehydrated whey but after adding sugar, stabilizer, flavor and citric acid to the fresh, separated whey, the whey solids content of the finished product was between 4 and 5%.

The whey sherbets were frozen most successfully in a continuous freezer. When a batch freezer was used excessive whipping occurred. Overrun in the batch freezer could be controlled by the addition of fat. A normal amount of citric acid was required for sherbet made from low acid whey, but no citric acid was needed with cottage cheese whey. The whey sherbets had a smooth body and texture, were more refreshing than other sherbets and had no whey flavor when made from a good quality whey.

W. H. Martin

920. Shrinkage. J. C. LANDO and C. D. DAHLE, Penn State College, State College. Ice Cream Trade J., 45, 10: 90, 114-115. Oct., 1949.

Of 12 commercially shrunken samples of ice cream studied, all but 2 showed a formal titration in excess of the titration for the control mix of the same nitrogen content, indicating that the shrunken ice cream protein had undergone some degree of change causing them to have a higher formal titration. A study was made of changes in the distribution of mix proteins when treated with the proteolytic enzyme, trypsin. Mix treated with 2 g. of trypsin to 45 lb. of mix and held at

40° F. for 24, 48, 72 hr. showed little or no shrinkage in the resulting ice cream after 1 wk. in a cabinet at -15° F. Shrinkage did result when the mix was held for 96 hr., indicating that some change was occurring during the 72 to 96 hr. period which was of major importance in enzymatic shrinkage.

Other tests were made to determine any change in protein distribution when ice cream was subjected to severe dry ice exposure, and also the effect of the albumen-globulin fraction of milk protein in ice cream shrinkage. Additions of the unaltered whey proteins to ice cream mixes reduced shrinkage considerably. W. H. Martin

921. Control of ice cream texture with microscope. W. S. ARBUCKLE, Univ. of Md., College Park. *Ice Cream Trade J.*, 45, 10: 86, 114. Oct., 1949.

The microscopical examination of ice cream may reveal body and texture characteristics which are not readily detected organoleptically. Smooth textured ice cream will have a large number of evenly distributed ice crystals and air cells. Coarse textured ice cream will have numerous large ice crystals along with fewer small crystals and less uniformity of crystals and air cells.

Microscopic examination is made by preparing a thin section of ice cream, imbedding the section in immersion oil and examining at a magnification of 100 times. This work usually is done at hardening room temperature. W. H. Martin

922. New frozen citrus purees and their uses. F. A. BEAVENS, Bureau of Agr. and Ind. Chem., U.S.D.A., Pasadena, Cal. *Ice Cream Trade J.*, 45, 10: 58, 96. Oct., 1949.

Successful processing of citrus fruit purees has been accomplished and provides fruit bases which possess natural flavor, color and nutritive value. These purees can be kept in good condition for a year when stored at 0° F. Use 14 to 18 oz. of 5 to 1 orange puree and 1.5 oz. of a 50% solution of citric acid solution to 1 gal. of sherbet mix, stir thoroughly, freeze at 50 to 65% overrun and then place in containers and harden. For lemon sherbet only 10 to 14 oz. of puree and 0.5 oz. of 50% citric acid are needed. The citric acid solution should be added after freezing when the sherbet mix contains any milk products to prevent curdling.

Milk sherbet mixes should contain 2.5% fat, 2.5% milk solids and 25% sugar. W. H. Martin

923. Gallonage analysis. Anonymous. *Ice Cream Trade J.*, 45, 9: 42, 88-89. Sept., 1949.

The International Association of Ice Cream Manufacturers has compiled an analysis of U. S. wholesale and retail ice cream production by manufacturers. The recently issued *Ice Cream Sales Index* for 1948 shows the number of wholesale ice cream manufacturers in the U. S. to be 3,766, producing 91.7% of all commercial ice cream. Of these 3,766, there are 2,879 which sell ice cream by wholesale only, with the remaining 887 retailing in their own stores, though they are primarily wholesalers. The 10,394 manufacturers who made and sold at retail only accounted for only 8.3% of the national production.

Of the industry's 629,090,000 gal. produced, the 3,766 wholesale manufacturers were responsible for 577,026,000 gal. and the 10,394 retailers for 52,064,000 gal. This report is substantiated by figures from the U. S. Department of Agriculture based on 1946 operation and by the U. S. Census of Manufacturers' report for 1947.

W. H. Martin

Also see abs. no. 900, 902, 909, 912.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

924. The fate of radioactive copper administered to the bovine. C. L. COMAR, G. K. DAVIS and LEON SINGER, Fla. Agr. Expt. Station, Gainesville. *J. Biol. Chem.*, 174, 3: 905-914. July, 1948.

Fifteen cattle were given an isotope of copper (Cu^{64}), orally in some cases and by jugular injection in others. The Cu retention was very low when fed but was highly retained when injected intravenously. In both cases, the Cu retained was widely distributed in the body tissues but more concentrated in the liver. A table showing the Cu distribution in the various body tissues is included.

A. O. Call

925. The transfer of immunity to the new-born calf from colostrum. E. L. SMITH and AUGUST HOLM, E. R. SQUIBB & SONS, New Brunswick, N. J. *J. Biol. Chem.*, 175, 1: 349-357. Aug., 1948.

Electrophoretic studies show that γ -globulin and T-globulin, both found in the serum of the mother, are not present in the serum of the new-

born calf or the new-born lamb, demonstrating that there is no placental transfer of antibodies in these species. This is in direct contrast to what is found in humans, where the γ -globulin in the serum of the new-born exceeds that of the mother. The calf acquires immunity through the ingestion of colostrum. A. O. Call

926. Passage of selenium through the mammary glands of the white rat and the distribution of selenium in the milk proteins after subcutaneous injection of sodium selenate. K. P. McCONNELL, Univ. of Rochester, Rochester, N. Y. J. Biol. Chem., 173, 2: 653-657. April, 1948.

Radioactive selenium was injected subcutaneously into lactating white rats and was shown to be present in the carcasses of the suckling pups within 24 hr., thus confirming previous reports of selenium being transmitted in the milk. To determine the milk fraction carrying the selenium, the stomach contents of 2 litters of rats were removed and the milk curd resuspended and fractionated. The protein fraction carried selenium. A. O. Call

927. Effect of the blood glucose level on the secretion of the adrenal cortex. G. L. STEEPLES and H. JENSEN, Medical Dep't., Field Research Laboratory, Ft. Knox, Ky. Am. J. Physiol., 157, 3: 418-421. June, 1949.

Studies were made on 225-280 g. rats. Adrenal glands were weighed, blood sugar measured and cholesterol content of the adrenal glands determined. Hyperglycemia, induced by glucose administered orally in a 50% solution, inhibited hormone release from the adrenal cortex as measured by the cholesterol content of the adrenal cortex. With the same measurement, hypoglycemia induced by insulin injections stimulated hormone release from the adrenal cortex.

How the blood sugar level influences the secretion of adrenal cortical hormones is not definitely known, although a plausible explanation is that the blood sugar level affects the secretion of

the pituitary adrenocorticotrophic hormone, which in turn regulates hormone secretion by the adrenal cortex. V. Hurst

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

928. Quaternary ammonium compounds in dairy sanitation. C. C. PROUTY, Dept. of Dairy Husbandry, State College of Washington, Pullman. Milk Plant Monthly, 38, 9: 46-48. Sept., 1949.

A discussion of present knowledge of Quaternary ammonium compounds is presented. The various phases covered are: (a) methods of determining germicidal efficiency, (b) physical reaction of bacteria in contact with quaternary compounds, (c) bacteriostatic action of the compounds, (d) the influence of pH, type of water and organic matter on the germicidal efficiency and (e) selective action on organisms.

J. A. Meiser, Jr.

929. Deposition of aerosol particles. A. II. YEOMANS, E. F. ROGERS and W. H. BALL, U.S. D.A., Agr. Research Adm., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., 42, 4: 591-596. Aug., 1949.

Tests with DDT aerosols determined the proportional deposition of toxicant on horizontal and vertical surfaces. Comparisons were made on the basis of (a) toxicity to insects, (b) chemical analysis, (c) visual observation of dyed deposits and (d) no. and size of particles.

Aerosol application in still air, simulating that in closed buildings, showed very little or no deposition on walls or other vertical surfaces. The particles settled almost solely upon the top of horizontal surfaces. Chemical analysis recovery of DDT from wall panels was less than 1% as great as from floor panels. Data on aerosol deposition in moving air, 2 to 16 mi./hr. also were included. E. H. Fisher

AUTHOR INDEX OF ABSTRACTS

- ABBOTT**, C. W., research in cheese industry, A162
- ABD-EL-MALEK**, Y., streptococci of milk, A64; staphylococci and micrococci of milk, A65
- ABELE**, C. A., can-washing, A117
- ACHAYA**, K. T., chemistry and mfg. of Indian dairy products, A59
- ACKERSON**, C. W., lactose determination, A166
- ADRIANAI**, W., firmness of summer butter, A46; texture of winter butter, A46; differences in firmness of summer butters, A47; factors influencing composition of milk fat, A71; crystallized fat in cream, A93
- AHLMANN**, S. W., fatty acids in cream and butter, A17
- AHMAD**, K., synthesis of vaccenic acids, A3
- ALBRECHT**, W. A., brucella infections, A151
- ALCORN**, H. A., teat draining tube, A6
- ALLEN**, N. N., colostrum feeding, A6; clipping cattle, A29
- ALLISON**, I., brucella infections, A151
- ALMY**, E. F., crude lactose, A107; stabilizing evaporated milk, A133; preparation of crude lactose, A163
- AMERDING**, G. D., reading Babcock test, A94
- ANDERS**, P. L., by-product sales by routemen, A172; sales bonuses for routemen, A184
- ANDERSON**, C. W., staining milk and cream films, A20
- ANDERSON**, E. A., butter cutter, A32
- ANDREWS**, D. B., packaging cheese, A106
- ANDREWS**, J., pasteurization and health, A19
- ANONYMOUS**, cooperatively-built milk plant, A5; electric dairy farm, A7; liquid sugar for ice cream, A7; container-dipper-cone combination, A9; driver-owned milk routes, A10; fried ice cream, A25; wind tunnel for hardening, A25; freon-refrigerated plant, A36; diced ice cream, A54; 1948 ice cream gallonage, A54; Sanders-Sager test, A71; ice cream eclairs, A79; 1948 ice cream gallonage, A80; non-fat fortified milk, A81; low-temperature cheese storage, A89; mechanical turning of cheese, A89; multiple-product dairy plant, A94; bowls for ice cream promotion, A99; ice cream sales, A99; milk delivery, A99; NaHCO₃ in homogenized cream, A100; milk samples by air express, A107; ice cream sales, A112; ice cream prices, A113; cottage cheese sales, A120; penicillin for mastitis, A130; milk is safe, A135; home ice cream market, A140; ice cream "snowballs," A140; home delivery of milk, A141; cabinet sizes, A145; ice cream specialty, A145; "Redi Serv" fountain, A145; sales and profits of malteds, A145; ice cream volume, A146; whipped cream in cans, A146; alfa continuous buttermaking process, A161; ice cream fleet refrigeration, A168; ice cream consumer survey, A170; flavor and texture in ice cream buying, A174
- ARBUCKLE**, W. S., low temperature dairy products' storage, A81; ice cream stabilizers and emulsifiers, A175; ice cream texture control, A187
- ARMERDING**, G. D., milk cans vs. tank trucks, A11
- ARMSTRONG**, G. B., dry ice refrigeration, A26
- ARRILLAGA**, C. G., dwarf cattle for the tropics, A154
- ARKOTT**, W., power costs, A138; safety program, A139; steam costs for heating, A169
- ASCHAFENBURG**, R., phosphatase test for milk, A166
- ASDELL**, S. A., variations in bovine vaginal smears, A174
- ASH**, B. A., bovine semen evaluation, A53
- ASHIE**, H. J., depreciation reserves, A53
- ASHWORTH**, U. S., whey denaturation by heat, A69; minimum heat treatment for whole milk powder, A162
- ASSENOWA**, K., universal agar medium, A91; counting milk bacteria, A134
- ASSOC. OFFICIAL AGRICULTURAL CHEMISTS, INC.**, *Methods of Vitamin Assay*, A181
- ATKESON**, F. W., Karakul-type curliness inheritance in Ayrshires, A185
- ATKINS**, M. E., carotene retention in alfalfa meal, A173
- BABCOCK**, C. J., effectiveness of milk ordinances, A10
- BABSON**, H. B., overhead support for milkers, A37; milking parlor, A154
- BADR**, A. A., green berseem, hay and silage, A153
- BAER**, R. A., work simplification, A125
- BAILEY**, G. F., carotene retention in alfalfa meal, A173

- BAILEY, G. L., L-thyroxine for cows, A146
BAKALOR, S., composition of South African milk, A67; mould growth in Roquefort cheese, A162
BAKER, D. T., thermophilic bacteria in milk, A33
BAKER, M. P., coliform bacteria in milk, A2; dairy farm sanitation, A15
BALL, W. H., deposition of aerosol particles, A188
BALSLEY, M. B., milk in cooked foods, A55
BAND, T. E., animal shelter research instrumentation, A144
BANKOWSKI, R. A., sulfamethazine for mammary gland infections, A45
BARKER, E. M., high quality cream, A82
BARKI, V. R., microorganisms in rat cecum, A84
BARLOW, F. S., painless dehorning, A78
BARNICOAT, C. R., keeping quality of butter, A105; free air in butter, A105; storage life of butter, A105
BARON, MARGARET, rheology of cheese, A106; statistics of cheese rheology, A106; ripening of Cheshire cheese, A162
BARRETT, G. R., dilution and spermatozoa fertility, A144
BARSANTINI, J. C., steroids and lactation, A13
BARTLETT, D. E., bovine venereal trichomoniasis controls, A44; treatment of *Trichomonas foetus* infection in bulls, A44
BARTLETT, S., L-thyroxine for cows, A146
RATCHELOW, A. B., hypochlorite destruction of *S. salivarius*, A49
BAUM, E. L., cutting operating cost, A5
BEACH, B. A., *Brucella abortus* vaccination, A159
BEARDSLEE, A. C., low-moisture powdered products, A90
BEAVENS, F. A., frozen citrus purees, A187
BECCHIA, H., centrifugal homogenizer, A184
BECKETT, D. C., extraneous matter in cheese, A106
BEISER, S. M., substance related to blood group B substance in bovine stomach, A150
BELL, C. J., refrigeration for ice cream manufacture, A51
BELL, E. J., Q fever in cattle, A103
BENDER, L. F., milking machine suspension, A78
BENDIXEN, H. A., whey denaturation by heat, A69
BERMAN, D. T., *Brucella abortus* vaccination, A159
BERNKOPF, H., bovine leptospira, A119
BERRY, A. E., ice cream sanitation, A85
BESSELL, C. J., dye reduction tests on pasteurized milk, A49
BETHKE, R. M., proteins and roughage digestion, A142; corn starch and roughage digestion, A153
BICKOFF, E. M., carotene retention in alfalfa meal, A173
BLACK, L. A., evaluation of quaternary germicides, A14; direct microscopic clump counts, A90; stains for direct microscopic examination of milk, A163
BLACKBURN, E. G., renal excretion of Ca by cow, A178
BLAUSER, L. P., milk cooling, A11
BLOCK, R. J., purifying casein, A109
BLUM, H., tote box, A100
BOBER, L. E., thermophilic bacteria in milk, A33
BOHSTEDT, G., vitamin A and C of sheep, A149; composition of sow's colostrum and milk, A154
BOLAND, H. A., fieldmen and milk quality, A117
BOLTON, W. D., procaine penicillin G for mastitis, A151
BORTREE, A. L., bacteriological examination of milk, A2
BOTHAM, G. H., corrosion by sodium hypochlorite, A180
BOUCHER, B. A., modern dairy plants, A72
BOWEN, J. F., lipolytic bacteria in Cheddar cheese, A105; rancid flavors in Cheddar cheese, A162
BOWIE, D. A., farm indications, A38
BOWLAND, J. P., composition of sow's colostrum and milk, A154
BOYD, J. C., resazurin test for milk, A32
BRANCHFIELD, F. B., cheese pusher, A47
BRASCH, A., electronic sterilization, A51
BREED, R. S., forty years of sanitation, A16
BREWSTER, BARBARA A., ash determination in lactose, A166
BREWSTER, D. A., ash determination in lactose, A166
BRIDGER, G. L., phosphatic animal-feed supplement, A143
BRIGGS, H. M., urea as protein supplement, A141
BROCH, GERR, vitamin A and carotene in Norwegian milk and butter, A100
BROOKS, R. F., cationic germicide activity, A34

- BROUWER, W., correction of butyrometer readings, A108
- BROWN, J. M., control in ice cream sales, A9
- BROWN, R. A., bovine serum protein fractionation, A150; fractionation of γ -globulin, A179
- BROWN, R. W., streptomycin in mammary gland, A119
- BROWNE, T. W., sulfamethazine for mammary gland infections, A45
- BRUHN, P. A., *Brucella abortus* ring test, A44
- BRUNNER, J. R., rough homogenized milk, A27; Babcock test of reconstituted milk, A71, A90; fat test for homogenized milk, A166
- BRYANT, L. R., yields of Cheddar cheese, A61; improved dairy indicator solution, A68
- BUMPUS, F. M., synthesis of vaccenic acids, A3
- BURGESS, A. F., DDT residue properties, A155
- BURGESS, J. W., milk fever, A17
- BURKHART, R. L., sulfamethazine blood concentrations, A57
- BURNETT, CLARON, ice cream vending machines, A37; cutting costs with pen barns, A97
- BURNIER, A., labor and industry development, A74
- BURROUGHS, W., proteins and roughage digestion, A142; soybean oil meal for cattle, A142; corn starch and roughage digestion, A153
- BURTON, K. A., carbon assimilation by yeasts, A66
- BUTTERFIELD, G. P., lactalbumin preparation, A50
- BUTZ, D., milk drying costs, A113
- BUXTON, J. C., group C streptococci in bovine mastitis, A160
- BUZZO, T. M., casein solution, A122
- BYRNE, J. V., sustaining penicillin levels, A57
- CADY, J. F., procaine penicillin G for mastitis, A151
- CALHOUN, J. R., casein solution, A122
- CALISE, V. J., feedwater treatment, A124
- CAMPBELL, B. A., dairy product prices, A76
- CAMPBELL, L. E., ultrasonic energy, A123
- CAMPBELL, M. M., bovine mastitis, A17
- CANHAM, A. S., *Fibrio foetus* infection, A31
- CANN, J. R., bovine serum protein fractionation, A150; fractionation of γ -globulin, A179
- CAPPS, B. F., bacteriological examination of edible fats, A165
- CARLYLE, G. M., milk distribution, A171
- CARNEY, P. E., thermoduric bacteria in milk, A33
- CARR, P. H., lactic streptococcus bacteriophages, A121
- CARSON, BARBARA L., vitamin D milk assay reports, A83
- CARRILLO, C., ring test for brucella agglutinins, A151
- CARTER, R., wearing rings in centrifugal pumps, A140
- CARTER, R. H., insecticide studies with dairy cattle, A97; DDT spray residues on foods, A150; DDT content of milk, A186
- CASIDA, L. E., dilution and spermatozoa fertility, A144; semen metabolism and fertility, A153
- CASLER, H. L., advantages of emulsifiers in ice cream, A8
- length of gestation, A114
- CASTLE, O. M., Jerseys in New Zealand, A114; CHAIKOFF, I. L., iodine compounds in fetal bovine thyroid, A148; thyroid gland growth, A148
- CHANDRA SEKAR, C. V., psychrophilic bacteria in milk, A34
- CHAPIN, E. K., dry egg composition, A163
- CHAPMAN, C. J., superphosphate in manure, A78
- CHARKEY, L. W., separation of coliforms, A122
- CHENG, P., dilution and spermatozoa fertility, A144
- CHILSON, W. H., milk quality tests, A175
- CHRISTIAN, J. A., carotene absorption and utilization, A153
- CHRISTIANSEN, M., ed., *C. O. Jensen. Selected Papers*. Vol. I., A159
- CHRYSLER, L. H., stabilizing evaporated milk, A133
- CLARKE, J., starters for Roquefort-type cheese, A132
- CLARY, P. D., JR., whey drying, A121; milk food for infants, A121
- CLAYDON, T. J., material in dust and debris, A30
- CLEMONS, H. B., Ca and P in cottage cheese, A71
- CLIFTON, C. E., ed., *Annual Review of Microbiology*, A31
- COFFIN, J. M., renal excretion of Ca by cow, A178
- COLLINS, W. F., storing milk solids, A25
- COLVARD, C. D., frozen concentrated milk, A121
- COMAR, C. L., retention of radioactive Ca by cattle, A187

- CONLEY, A. R., history of dairy research, A57
 COOK, E. T., pancreatic diabetes of the calf, A149
 COOK, ROBERT, bovine quintuplets, A78
 COPEMAN, L. C., device for removing cream from bottle, A176
 CORASH, P., thermotolerant bacteria in milk, A33
 COSTILON, R. N., cultured buttermilk, A107
 COULTER, S. T., packaging of print butter, A45
 COUNCIL ON FOODS AND NUTRITION, vitamin A and D fortification of milk, A28
 COURTNEY, J. L., plate count ratio studies, A165
 COUSINS, C. M., pH of sterilizing solutions, A29
 COWIE, A. T., adrenal cortex and mammary glands, A14; pregnancy diagnosis tests, A59
 COX, G. A., properties of New Zealand butters, A70
 CRAIG, A. H., JR., renal excretion of Ca by cow, A178
 CRAWFORD, C. W., insecticides and the food law, A155
 CREWS, J. F., centrifugal atomizer, A137
 CROSSLEY, E. L., keeping quality of pasteurized cream, A82
 CROWLEY, F. L., frozen homogenized milk, A128
 CUNKELMAN, J. W., bovine mastitis, A152
- D**
 DAHLBERG, A. C., sanitizing milking machines, A15; flavor in Cheddar cheese, A61; ice cream as food, A83
 DAHLE, C. D., storing milk solids, A25; viscosity in chocolate ice cream, A99; ice cream defects, A115; good ices and sherbets, A175; ice cream defects, A175; ice cream shrinkage, A186
 DAVEY, W. R., viscosity in chocolate ice cream, A99
 DAVIDSON, W. M., urea as protein supplement, A126; isotope-labelled urea, A126; synthesis of labelled urea, A126; digestibility of barley, A173; digestibility of linseed oil meal, A173
 DAVIES, D. V., fructose and citric acid in seminal glands, A173
 DAVIES, E. P., sterilization of milk bottles, A100
 DAVIES, F. L., JR., heat resistance and germicide resistance, A65; selection and heat resistance of spores, A65
 DAVIS, G. K., retention of radioactive Cu by cattle, A187
 DAVIS, J. G., dye reduction tests on pasteurized milk, A49
 DAVIS, J. L., seasonal conception rates, A174
 DEANE, H. W., alkaline phosphatases, A147
 DECKER, C. W., butteroil observations, A152; plastic cream and butteroil, A163
 DEGLER, H. E., water-cooling tower upkeep, A167
 DEMAN, TH. J., organic acids for acidifying skim milk, A185
 DEMMLER, G., whey quality, A48
 DEMPSEY, E. W., alkaline phosphatases, A147
 DEN HERDER, P. C., diacetyl in butter, A108
 DERBY, J. V., JR., carotene determination methods, A23
 DETWEILER, D. K., Sulkowitch test for bovine hypocalcemia, A178
 DEUEL, H. J., JR., alkaline phosphatases, A147
 DEUTSCH, H. F., whey proteins, A109; immune globulins, A110
 DEUTSCH, J. J., ice cream scoop, A38
 DEVOR, J. R., chocolate coated ice cream, A7
 DEWITT, J. B., carotene determination methods, A23
 DICKE, R. J., *Hypoderma* larvae in bovine spinal canal, A161
 DIESEL, A. M., east coast fever in S. Africa, A31
 DIKMANS, G., bovine venereal trichomoniasis controls, A44
 DINNING, J. S., urea as protein supplement, A141
 DOAK, A. C., ice cream cabinets, A80
 DOAN, F. J., increasing cream viscosity, A27
 DODD, F. H., milking techniques, A176
 DODDANANJAYYA, R., Q fever in eastern Washington, A181
 DORTSCH, R. N., microbacteria, A65; *Elementary Experiments in Dairy Bacteriology*, A129
 DOLAN, P. J., JR., checking HTST equipment, A137
 DONALDSON, R. W., benzene hexachloride-flavored milk, A27
 DONEAM, C. B., pathogenicity of *Brucella suis* for cattle, A44
 DONNELLY, D., spray cooler for milk cans, A39
 DOUGHERTY, F. C., carotene absorption and utilization, A153
 DOWLING, D. F., extracting fertilized bovine egg, A144

- DOYLE, H., milk acidity studies, A67; modifications of the Kjeldahl method, A68
- DUMMETT, G. A., corrosion by sodium hypochlorite, A180
- DUNCAN, C. W., amino acids of bovine semen, A114
- DUNCAN, G. R., milk can transport, A127; milk cooler, A183
- DUNKLEY, M. E., milk treatment with oxidation-inhibiting gases, A93
- DUNLOP, H. G., liquid sugar in ice cream, A7
- DUNN, M. S., chromatography of amino acids, A137
- DYE, J. A., alloxan administration, A149; pancreatic diabetes of the calf, A149
- DYREDAHL, SVEN, feeding iodinated casein, A130
- E**ADES, M. K., milking machine shut-off, A115
- EASTON, H. J., support for milker tubes, A153
- EATON, H. D., carotene absorption and utilization, A153
- EBERT, H. G., trap discharge lines, A139
- EDDY, G. W., residual insecticides for flies, A155
- EDGINGTON, B. H., proteins and roughage digestion, A142; corn starch and roughage digestion, A153
- EDWARDS, M., high colony counts in pasteurized milk, A91
- EIGEL, G., microflora and structure of Camembert cheese, A18
- EILERS, H., chemistry and physics of dairy products, A157
- ELDRIDGE, F. E., Karakul-type curliness inheritance in Ayrshires, A185
- EL-KATIB, M. T., green berseem hay and silage, A153
- ELLIKER, P. R., *Practical Dairy Bacteriology*, A157; pasteurization effect on microorganisms, A166
- ELLSWORTH, P. R., mechanical milker clinic, A127
- EL-SADEK, G. M., microbial action on butterfat, A165
- ELVEHJEM, C. A., microorganisms in rat cecum, A84
- ENGEL, CHE., nutritive value of milk, A116
- ENGEL, E. R., whey fermentation method, A21
- ENGLAND, C. W., quality in manufacturing ice cream, A80
- ENGLE, R. W., selling at collection time, A52
- ENSMINGER, M. E., residual arsenic and strychnine, A116
- ERB, R. E., bovine semen evaluation, A53; breeding failure survey, A53; calf raising facilities, A54; blood ascorbic acid, A57
- ERDMAN, F. S., *Principles of Food Freezing*, A43
- ERICHSEN, I., growth of thermotolerant bacteria in cheese, A65
- ERICKSON, C., scraper for freezers, A122
- ERSHOFF, B. H., nutritive value of butter, A147
- ESPINASSE, G. H. G., ice cream freezer, A137
- ESPINASSE, J. P. C., ice cream freezer, A137
- ETCHELLS, J. L., potato silage fermentation, A66
- EVAN, E. J., incidence of thermotolerant organisms, A91
- EVAN, W. C., marsh ragwort as animal poison, A152
- EVANS, B. G., air filter maintenance, A138
- EVANS, F. T. R., marsh ragwort as animal poison, A152
- F**ACKELMEIER, K., whey beverage, A48
- FANWALD, L., collecting slow accounts, A184
- FARRAG, H., penicillin sensitivity of animal staphylococci, A160
- FAUST, H. P., can washers, A117
- FAUVET, E., regulation of lactation, A28
- FELLERS, C. R., preserving effect of organic acids, A21
- FERGUSON, E. J., pasteurizing and cooling cream, A60; efficiency in plant operation, A74
- FERRAZ, N., collecting liquid sediments, A100
- FERRETTI, A., artificial textile fibers, A3
- FERRIS, L. W., milk fat in milk chocolate, A27
- FINK, H., proteins of kefir fungi, A56
- FINNEBURCH, M. L., ice cream sales, A112
- FISCHER, O., checking short time heating by phosphatase test, A21
- FISHER, F. M., process cheese, A133
- FLAGG, H., cottage cheese sales, A126
- FLEMING, D., epidemiology of brucellosis, A159; human and bovine brucellosis, A159
- FLOHR, H., JR., cattle restraining device, A54
- FOHRMAN, M. H., growth of crossbred dairy heifers, A174
- FOLEY, E. J., sustaining penicillin levels, A57
- FOLLEY, S. J., adrenal cortex and mammary

- glands, A14; acetate as a possible milk-fat precursor, A83; L-thyroxine for cows, A146
- FOOT, A. S., milking techniques, A176
- FORBES, B. P., making chocolate milk, A12
- FOSTER, E. M., quaternaries vs. cholamine, A85
- FOSTER, T. L., oxidized flavors, A122
- FOUET, D. L., proved sire program, A53
- FOUST, W. L., fat-free milk, A38
- FRANKLIN, P. M., seasonal incidence of thermophilic organisms, A91
- FRAYER, J. M., procaine penicillin G for mastitis, A151
- FREAR, D. E. H., determination of DDT in milk, A3
- FRENCH, C. E., ice cream gallonage shifts, A145
- FRENCH, T. H., acetate as a possible milk-fat precursor, A83
- FRIEDEL, F. A., whey filtration, A133
- FRIEDGEN, A. E., delivery cost control, A125
- FRIETZ, W., butter structure, A31; physical aspect of butter, A32; mechanism of milk secretion, A39
- FUCHS, A. W., pasteurization and health, A19; U.S.P.H.S. milk code amendments, A38
- FULLER, R. W., sulfamethazine and penicillin for mastitis, A151
- FUNCK, E., historical data on whey and milk sugar, A48
- G**AHAN, J. B., DDT resistance of flies, A155
- GALLUP, W. D., urea as protein supplement, A142
- GARRETT, O. F., preparation of crude lactose, A163
- GAUMNITZ, E. W., future of cheese industry, A61
- GELLMAN, NAOMI, determining iron content of milk, A182
- GERLAUGH, P., proteins and roughage digestion, A142; soybean oil meal for cattle, A142; corn starch and roughage digestion, A153
- GERSDORFF, W. A., synthetic pyrethrin toxicity for flies, A155
- GHONEIM, A., green berseem, hay and silage, A153
- GHOSH, D., metabolic regulator in spermatozoa, A143; semen metabolism and fertility, A153
- GIBLER, J. W., milk-turning machine, A49
- GIBSON, C. A., bitter flavor in Cheddar cheese, A61; lipolytic bacteria in Cheddar cheese, A105; rancid flavors in Cheddar cheese, A162
- GIBSON, T., streptococci of milk, A64; staphylococci and micrococci of milk, A65
- GILBE, L., vitamin A and carotene in Norwegian milk and butter, A100
- GILCHRIST, L. J., ice cream packer, A54
- GILDEN, R. O., calf raising facilities, A54
- GLABAU, C. A., MSNF and bread quality, A163; MSNF in bread, A163
- GLAZIER, L. R., cream defects, A27
- GLEASON, T. A., inverter for milk cans, A117
- GOLDING, N. S., grading milk with resazurin test, A2; growth of thermophilic bacteria in cheese, A65; *P. roqueforti* in blue cheese, A66; starters for Roquefort-type cheese, A132
- GOLDSCHMIDT, E., dye reduction tests on pasteurized milk, A49
- GOODWILLIE, D. B., concentrated milk for ice cream, A63
- GORDON, G. E., bull costs, A24
- GORINI, C., acidobacteriolytic bacteria, A34; morphology and cultural characteristics of lactic acidoproteolytic cocci, A164
- GORTNER, W. A., *Principles of Food Freezing*, A43
- GOULD, I. A., fat variations in milk, A82
- GOULD, J. H., ice cream cost system, A170
- GOULD, J. S., ice cream emulsifiers, A79
- GRAVES, F. W., pen stable experience, A37
- GRAY, J. A., serum mineral content, A143
- GREEN, G., deflocculating bacteria, A134
- GREENSPAN, J., producing devitaminized casein, A152
- GREEP, R. O., alkaline phosphatases, A147
- GREGOIRE, C., goitrogenic effect of aminothiazole, A178
- GREGORY, H. W., 4-day cream delivery, A105
- GREGORY, J. M., municipally-owned milk plant, A10
- GREGORY, P. W., hereditary digital anomaly, A154
- GRIFFITHS, D., sterilization of milk bottles, A100
- GRINDROD, G. E., mineral-fortified milk product, A182; fortified concentrated milk, A183
- GRINDROD, G. G., canning evaporated milk, A163
- GROCOFF, W., butter cutter, A161
- GROOT, J., factors influencing the composition of milk fat, A70

- GRUBEN, S., egg substitutes, A109
GRUMMER, R. R., composition of sow's colostrum and milk, A154
GUNDERSON, N. O., staining milk and cream films, A20
GUTHRIE, F., brucella isolation, A121
GUTHRIE, J. E., solution for dehorning calves, A6
GUYMON, J. F., triangular taste tests, A58
- H**
HABERMAN, W. O., *Hypoderma* larvae in bovine spinal canal, A161
HADJIDIAN, Z., hyaluronidase activity of spermatozoa, A6
HADLEY, F. B., *Principles of Veterinary Science*, A129
HADZI-ANTIC, A., degeneration of lactic acid streptococci, A92
HALL, J. I., temperature control for pasteurizers, A94
HALL, J. M., spray drier apparatus, A122; process for dehydrating milk, A183
HALL, S. H., can hoist, A153
HAM, W. E., residual arsenic and strychnine, A116
HAMMER, B. W., butter manufacturing process control, A111; butter manufacturing process, A111
HAMMOND, E. V., chocolate dairy drink, A176
HANCOCK, J. R., *Corynebacterium pyogenes* A144
HANCOCK, J. R., *Corynebacterium pyogenes* in bull semen, A78
HANSEL, W., variations in bovine vaginal smears, A174
HANSELL, O. F., mechanical cheese punch, A48
HANSEN, H. C., resazurin test for milk, A32
HANSEN, O. S., emulsifying agent for ice cream, A98
HANSEN, R., heat destruction of milk phosphatase, A93
HANSEN, R. G., blood serum proteins of calves, A110; colostrum pseudoglobulin, A110
HANSON, A. M., enhancing yield of yeast from whey, A93
HANSSON, E., rennet coagulum, A108
HAPPY, F., denaturation of casein fibers, A167
HARDIN, L. S., *Farm Work Simplification*, A103
HARDING, H. G., detergent evaluation, A40
HARRISON, C. M., forage crop management, A24, A77
- HARSTICK, W. H., supply tank for centrifugal separator, A183
HART, E. B., tocopherols and milk production, A114
HART, G. H., motility of spermatozoa, A6
HARTSELL, S. E., pathogens in frozen eggs, A92
HÄSING, J., butterfat determination, A135
HAUCK, G. W., piping operating costs, A169
HAUG, H., self-serving ice cream, A140
HAWLEY, H. B., bacteriology of sweetened condensed milk, A49
HECHTER, O., hyaluronidase activity of spermatozoa, A6
HEDRICK, T. I., manufacture of milk fat, A18
HEIDENREICH, T. E., JR., dealer profits in ice cream, A26; ice cream in theaters, A140
HEIM, H. C., germicidal value of glycols, etc., A34
HEINTZ, O. E., supply tank for centrifugal separator, A183
HELM, R., paratyphoid and enteritis bacteria in whey, A49
HEMKER, A. H., livestock watering device, A174
HENDRIX, B. M., pancreatin digestion of acetyl proteins, A136
HENRARD, F. J. J. J., metallic churn and butter kneader, A182
HENRY, KATHLEEN M., deterioration of dried skim milk, A62; dried skim milk in bread, A177
HERMAN, H. A., roughage and heifer growth, A142
HERREID, E. O., composite milk samples, A3
HERRINGTON, B. L., *Milk and Milk Processing*, A43
HESS, E. L., immune globulins, A110
HETRICK, J. H., advance in dairy technology, A12
HEYMAN, A. A., ice cream cup, A115
HIBBS, R. A., partial homogenization of ice cream mix, A25
HICKS, T. G., purging refrigeration, A138
HIGGINBOTTOM, CONSTANCE, bacteriological quality of spray-dried milk powder, A62; bacterial growth in reconstituted dry milk, A66; dye-reduction tests for dried milk, A66
HILDER, R. A., growth of crossbred dairy heifers, A174
HILL, D. C., yields of Cheddar cheese, A61
HILLIG, F., butyric acid in cream, A18

- HILLIG, J., fatty acids in cream and butter, A17
- HIRSCH, A., inhibitory streptococci, A20
- HITES, B. D., lactose determination, A166
- HOBBS, N. L., bacteriological examination of edible fats, A165
- HOBSON, P. M., seasonal incidence of thermophilic organisms, A91
- HODSDON, F. G., pressure releaser milker, A54; can lifting device, A167
- HODSON, A. Z., folic acid activity, A136
- HOFFMAN, H. J., serum tests for added water, A185
- HOFFMAN, R. A., fumigating property of insecticides, A155
- HOLGREN, E. W., truck moisture problem, A26
- HOLLAND, R. F., HTST holding time, A51
- HOLM, A., immunity transfer by colostrum, A187
- HOLM, W. K., universal milk bottle plan, A11
- HOLMES, A. D., percheron's colostrum, A109
- HOLMQUIST, N., cows heat house, A186
- HOOD, E. G., bitter flavor in Cheddar cheese, A61; lipolytic bacteria in Cheddar cheese, A105; penicillin action on starters, A107; penicillin and cheese cultures, A134; rancid flavors in Cheddar cheese, A162
- HOPKINS, E. W., dried protein products, A121
- HOPF, H. C., process cheese, A138
- HOPSON, G. H., thermophilic bacteria in milk, A38
- HOPWOOD, J. A., milk delivery tube, A27
- HORNEMAN, H. C., butter manufacturing process, A111; butter manufacturing process control, A111
- HORWITZ, W., cheese analysis, A133
- HOWARD, H. W., purifying casein, A109
- HOWSE, C. G., mechanical milker, A127
- HOYLE, MARGERY, inhibitory strains of lactic streptococci, A63; identity of starter streptococci, A64
- HOYT, L. W., milk plant operation, A52
- HUBEL, J., milk truck refrigeration units, A168
- HUCKER, G. J., cationic germicide activity, A34; detergents as cleaner-sanitizers, A40; combined cleaner-sanitizing agents, A179
- HUDDLESON, I. F., sulfonamides action on brucella antibody-complement system, A8; ring test for brucella agglutinins, A151
- HUGHES, B. S., entrainment separator, A4
- HUGHES, D. L., results of mastitis survey, A45
- HUGHES, J. S., serum vitamin A, A113
- HULL, M. E., crude lactose, A107
- HUMPHREY, G. F., fructose and citric acid in seminal glands, A173
- HUMPHRIES, E. L. E., Frost little plate technique for milk, A134
- HUMPHRIES, E., mix cooling, A37
- HUNTER, G. J. E., morphology of *Streptococcus cremoris*, A134; effect of penicillin on lactic streptococci, A164
- HUNZIKER, O. F., *Condensed Milk and Milk Powder*, A119
- HURD, F. E., freeze drier, A137
- HURST, V., thiouracil and mammary growth, A128
- HUSSONG, R. V., butter manufacturing process, A111; butter manufacturing process control, A111
- HUTCHINGS, L. M., pathogenicity of *Brucella suis* for cattle, A44
- HUTCHINSON, R. C., *The Better Utilization of Milk*, A158
- HVIDSTEN, H., vitamin A and carotene in Norwegian milk and butter, A100
- HYAM, J. F., refrigeration efficiency, A138
- IBSEN, H. L., Karakul-type curliness inheritance in Ayrshires, A185
- Industrial Engineering Chemistry, symposium on food technology, A41
- IRVINE, D. M., efficient cheese factory operation, A74
- IRVINE, O. R., cutting cheese for retail, A61; yields of Cheddar cheese, A61
- ITZEROTT, A. G. F., milk pasteurization, A55
- JAMIESON, M. C., cleaner dairy equipment, A117
- JANSSEN, H. J. J., fat balance sheet, A112
- JENSEN, H., blood glucose level and secretion of adrenal cortical hormones, A188
- JERGENS, A. N., homogenizer, A72
- JOCHIMSEN, H., tote box, A100
- JOHNS, C. K., chemical sterilizers, A118
- JOHNSON, A., machine for cooling cream, A167
- JOHNSON, G. R., efficient dairy plant, A73
- JOHNSON, M. J., partition chromatography for fatty acids, A182
- JOHNSON, O. M., production costs, A172
- JOHNSON, R. B., renal excretion of Ca by cow, A178
- JOHNSTON, F. A., determining iron content of milk, A182

- JOHNSTON, J. F., choosing a package boiler, A169
- JOHNSTON, R. F., estrogen treatment, A147
- JOINER, C. R., sediment tests for milk and cream, A150
- JONES, G. E., sterility of washed milk cans, A101
- JONES, I. C., inheritance of Shorthorn colour, A77
- JONES, I. D., potato silage fermentation, A66
- JONES, J. P., paper bottle top, A27; cream dispenser, A54; paper bottle, A55
- JONES, L. B., thermoduric microflora of dairy utensils, A33; thermoduric and thermophilic bacteria, A49; incidence of thermoduric organisms, A91
- JONES-EVANS, E., thermoduric microflora of dairy utensils, A33
- JORDAN, W., HTST holding time, A51; measurement of pasteurization holding time, A72
- JOSEPHSON, D. V., chemical sterilizers, A15
- JUNK, W. B., liquid sugar in food products, A37
- JUNQUEIRA, P. B., effect of food supplements, A56
- K**
- KABAT, E. A., substance related to blood group B in bovine stomach, A150; *Experimental Immunochemistry*, A159
- KAHLER, D., high temperature dishwashing, A101
- KARASSIK, I. J., wearing rings in centrifugal pumps, A140
- KARUSH, F., bovine serum albumen, A110
- KASCHULA, V. R., *Treponema theileri* transmission, A31
- KASTELIC, J., tocopherols and milk production, A114
- KATZNELSON, H. K., penicillin action on starters, A107; penicillin and cheese cultures, A134
- KEIL, H. L., cheese making process, A18
- KELLEY, W. R., *Corynebacterium pyogenes* in bull semen, A78
- KELLOGG, W. D., packaging ice cream, A115
- KELLY, C. F., animal shelter research instrumentation, A144
- KEMKES, B., milk hygiene, A20
- KEMPER, R. D., milk can cooler, A167
- KENDALL, I. H., can-handling device, A137
- KENDALL, K. A., soil fertilization and lactation response, A113
- KENNEDY, E. M., frozen confection, A127
- KENNEDY, J. W., isotope-labelled urea, A126; urea as protein supplement, A126; digestibility of barley, A173; digestibility of linseed oil meal, A173
- KENTIE, A., growth-promoting factor in butter, A116
- KERN, H. F., JR., dispensing washing powder, A16
- KERR, C. A., improvements for creameries, A73
- KEYS, A., milk cholesterol, A21
- KIECHLIN, A. C., business worth, A5; minimizing tax expense, A23; adequacy of investment return, A75; depreciation reserves, A124
- KILLICK, C. H. P., cream improvement, A105
- KING, H. H., carotene concentrates, A77
- KING, N., determination of acetylmethylcarbinol plus diacetyl in butter cultures, A2; butter structure, A31; physical aspect of butter, A32; globular and free fat in butter, A88; fat globules in butter, A104; globular fat in butter, A104; structure of normal and continuous-process butter, A181
- KING, W. V., DDT resistance of flies, A155
- KINNEY, A. M., homogenizer, A72
- KIRKWOOD, J. G., bovine serum protein fractionation, A150; fractionation of γ -globulin, A179
- KISER, J. S., aureomycin for staphylococci mastitis, A151
- KLEIBER, M., secretion of P_{22} in milk, A128
- KLEMM, DOROTHY E., α -methylglucoside fermentation, A122
- KLINE, E. A., residual arsenic and strychnine, A116
- KLINZING, A. F., stock watering fountain, A154
- KLOTZ, I. M., binding properties of proteins, A136
- KLUGER, M. J., "break-even" point for milk plants, A141
- KNIGHT, R. L., genetic evolution of economic characters, A78
- KNIGHT, S. G., molds in dairies, A107
- KNIGHT, T., routeman incentives, A53; undercoating truck bodies, A86; routeman incentive plan, A113; sales contests for cottage cheese, A126; plant accessories, A127; milk sales contests, A171; rewarding routeman, A171
- KNUSDON, LULA, cheese analysis, A133
- KOBLER, P., epidemiology of brucellosis, A159
- KOERVER, C., ice cream flavors, A7, A79

- KOLLER, E. F., milk drying costs, A113
 KON, S. K., deterioration of dried skim milk, A62; dried skim milk in bread, A177
 KORPRICH, H., heritable factors of Brown Swiss bulls, A24; residual nitrogen of cow's milk, A69
 KOSIKOWSKY, A. C., flavor in Cheddar cheese, A61
 KRAFT, M. E., age and mastitic infection, A104; antibacterial agents for mastitis, A130
 KRAUSE, M. N., flow of milk cooling water, A167
 KREJCI, L. E., polysaccharide related to blood group substances, A149
 KRIENKE, W. A., partial homogenization of ice cream mix, A25
 KRUEGER, G. J., whey denaturation by heat, A69
 KRUISHEER, C. I., acidity of milk, A50
 KRUKOVSKY, V. N., stability of milk fat, A71
 KUMETAT, K., whey quality, A48; analysis of Milone, A63
 KUPPERS, J. R., nutritional variations in pasture grasses, A6
 KURZROK, R., hyaluronidase of rat testes, A14
- L**
 LACTRONE, G.m.b.H., nutritional evaluation of milk products, A56
 LAKRITZ, D. E., concentrated citrus oils, A79
 LAMBERT, J., thermoduric bacteria in milk, A33; thermoduric counts, A91
 LAMPERT, L. M., *Milk and Dairy Products*, A17
 LANCASTER, R. J., pasture digestibility estimation, A77
 LANDO, J. C., ice cream shrinkage, A186
 LANGER, P. H., sulfamethazine blood concentrations in calves, A57
 LARDY, H. A., metabolic regulator in spermatozoa, A143; semen metabolism and fertility, A153
 LAUGHLIN, M. E., digestible energy of whey, A173
 LAWRENCE, A. J., rennet casein for glue, A48
 LAWRENCE, J. G., lead halter for cattle, A54
 LAWRENCE, J. M., retail milk delivery, A75
 LEA, C. H., deterioration of dried skim milk, A62; determination of free amino-nitrogen, A68; reaction between dry milk protein and reducing sugar, A69
 LEE, D. H. K., bovine reactions to hot atmospheres, A84
 LEE, S. B., review on fermentation, A21
 LEE, S. W., sustaining penicillin levels, A57
 LEESMENT, A., counting bacteria, A90
 LEGGATT, A. G., quaternary ammonium compounds for creamery churns, A85; bacterial discoloration of butter, A165
 LEITCH, L. C., synthesis of labelled urea, A126
 LEMMEL, P. T., sealing empty cans, A121
 LEONARD, S. L., hyaluronidase of rat testes, A14
 LEROI, D., electronic pasteurization, A72; hydroponics, A77
 LEVINE, A. S., preserving effect of organic acids, A21
 LEVINE, B. S., direct microscopic clump counts of pasteurized milk, A90
 LEVINE, H. B., *Brucella abortus* 19 identification, A134
 LEVINE, S. B., stains for direct microscopic examination of milk, A163
 LEVITON, A., separation of proteins from milk products, A69; methanol extraction of skim milk powder, A135
 LEWIS, C., wages for milk routemen, A141
 LEWIS, T., sterilization of milk bottles, A100
 LI, J. C. R., dependability of food judges, A58
 LIEBERT, J. L., gassy fermentation in process cheese, A88
 LIGHTNER, D. C., tank trucks for milk, A146
 LINDEWALD, W., egg substitutes, A109
 LINDGREN, H. O., method of producing butter, A72
 LINDQUIST, A. W., fumigating properties of insecticides, A155
 LINDQUIST, H. G., benzene hexachloride-flavored milk, A27
 LINDREN, H. O., teat cut, A145
 LIPSETT, M. B., vitamin A and thyroid function, A14
 LITTLE, R. B., bovine leptospira, A119
 LOCHRY, H. R., low-acid rennet-type cottage cheese, A1
 LOFGREEN, G., carotene retention in alfalfa meal, A36
 LOOSLI, J. K., carotene retention in alfalfa meal, A36; placental and mammary tocopherol transfer, A114; tocopherol transfer, A143; tocopherol deficiency of sheep, A154
 LORENZEN, C., Jr., animal shelter research instrumentation, A144
 LOVVORN, R. L., phosphate fertilization, A143
 LOWENSTEIN, M., ascorbic acid and oxidized flavor, A22
 LUCAS, E. R., dealer education, A52
 LUCAS, P. S., ice cream flavor preferences,

- A26; Babcock test of reconstituted milk, A71; preferred ice cream flavors, A79; Babcock fat test of reconstituted milk, A90; fat test for homogenized milk, A166
- LUCK, J. M., ed., *Annual Review of Biochemistry*, vol. XVIII, A158
- LUECKE, R. W., amino acids of bovine semen, A114
- M**
- MAGOFFIN, R. L., epidemiology of brucellosis, A159
- MALCOLM, J. F., bovine mastitis, A17
- MALLMAN, W. L., high temperature dishwashing, A101
- MANN, H. D., DDT content of milk, A186
- MANN, T., fructose and citric acid in seminal gland, A173
- MANNING, MARY C., penicillin ointment for mastitis, A160
- MARKSTEIN, D., cost reduction for milk plants, A171
- MARR, A., milk fever, A17
- MARSELLI, E., shaft alignment check, A170
- MARTIN, ETHEL A., nutrition research in the dairy industry, A83
- MARTIN, J. E., Sulkowitch test for hypocalcemia, A178
- MARTIN, W. H., dehydrated sweetened condensed skim milk, A19, A90; superheating ice cream mixes, A25
- MARUYAMA, G. M., lactation studies, A116
- MASBRUCH, H., bull halter and controller, A54
- MASSON, G. M. C., steroids and lactation, A13
- MASTERMAN, NANCY K., *Principles of Food Freezing*, A43
- MATHEE, E. J., dealer education, A52
- MATRONE, G., phosphate fertilization, A143; milk diets for pigs, A154
- MATSON, W. E., calf raising facilities, A54
- MATTERSON, L. D., carotene absorption and utilization, A153
- MATTHEWS, J. I., estrogen and progesterone treatment, A146
- MAYNARD, L. A., dietary allowances, A55
- MAXWELL, M., seasonal production bonus, A76
- MAYER, M. M., *Experimental Immunochimistry*, A159
- MCAULEFFE, H. D., ice cream plant laboratory, A9
- MCCABE, J., fuels and firing, A96; fuels and firing, A123; flue dust problems, A168; heating buildings, A184
- MCCANDLESS, E. L., alloxan administration, A149; pancreatic diabetes of the calf, A149
- MCCONNELL, K. P., mammary secretion of selenium, A188
- MCCORD, C. P., odors, A60
- MCCULLOCH, E. C., aureomycin for staphylococci mastitis, A151
- MCDIARMID, A., avirulent brucella strains, A131; brucellosis immunization, A131
- MCDONALD, G. W., manufacture of rennet paste, A182
- MCDOWALL, F. H., properties of New Zealand butters, A70
- McFADDEN, J. B., cream separator and homogenizer, A35
- MCGINTY, D. A., antithyroid activity by ergothioneine, A148
- MCGREGOR, W. S., residual insecticides for flies, A155; horn fly control, A185
- MCINROY, E. E., autoclaving casein, A84
- McKENZIE, D. A., thermophilic bacteria in milk, A33; farm conditions, A38; procedure for thermophilic counts, A91
- McKINNEY, L. L., stabilized protein-formaldehyde dispersion, A69
- McLEOD, H. M., JR., phosphatic animal-feed supplement, A143
- McLEOD, W. G., cleaner dairy equipment, A117
- McMURRAY, A. A., optical method for mastitis milk, A87
- McNUTT, S. H., metrorrhagia in virgin heifer, A13
- MCVAY, L. V., brucella isolation, A121
- MEAD, S. W., hereditary digital anomaly, A154
- MEADE, R. E., enhancing yield of yeast from whey, A93; whey food, A120; milk food for infants, A121; whey drying, A121; centrifugal atomizer, A137
- MEEHAN, J. H., competition in ice cream sales, A140
- METTER, J., restricted feed intake, A147
- MELNICK, D., heat-processing and protein qualities, A177
- MENEZ, CAROLINE B., cottage cheese types, A132
- MERISH, F., goodwill, A52; price and profit, A74; balanced business, A124; sales contests, A126; accounting charts for milk plants, A171; cost control for dairy plants, A184
- MERRILL, D. G., food heating rates in glass, A36
- MESIER, J. A., JR., storing milk solids, A25
- METCALF, DOROTHEAE, cationic germicide activity, A34

- MEYER, E. A., RMA and dairy research, A58
 MEYER, J., pasteurization-control instruments, A137
 MEYN, A., microbial taxonomy, A134
 MICHELSON, I. D., brucella isolation, A121
 MICKELSEN, O., milk cholesterol, A21
 MIGAKI, H., aureomycin for staphylococcal mastitis, A151
 MILK INDUSTRY FOUNDATION, *Laboratory Manual*, A59
 MILLER, D. D., control of mold, A66; *P. roqueforti* in blue cheese, A66
 MILLER, P. P., ice cream serving costs, A184
 MILLER, R. C., paper bottle acceptance, A11; customer solicitation program, A52; efficient fleet maintenance, A172
 MILNES, BETSY J. C., milk fever, A17
 MINSTER, C. H., ammonia refrigeration, A73, A111, A123; cooling dairy products, A183
 MINTHORN, C. M., pasteurization of ice cream mixes, A98
 MITCHELL, H. L., carotene concentrates, A77
 MITTEN, H. L., JR., milk plant layout, A72
 MOHRING, R., staining milk and cream films, A20
 MOHR, W., fat globule deformation in cream, A48; viscosity of high fat cream, A71; body of butter, A88; butterfat determination, A135
 MONROE, R. A., oral effectiveness of thyroxine forms, A148
 MOORE, A. V., quaternary sanitizers and milk quality, A15; home milk pasteurizer, A81
 MOORE, J. W., phosphatic animal-feed supplement, A143
 MOORE, L. A., insecticide studies with dairy cattle, A97
 MORGAN, B. B., metrorrhagia in virgin hoifer, A13; *Hypoderma* larvae in bovine spinal canal, A161
 MORGAN, K. J., sterilization of milk bottles, A100
 MORGAN, O. M., synthetic detergents, A128
 MORRIS, C. S., high colony counts in pasteurized milk, A91
 MORRISON, M., thermotolerant bacteria in milk, A33
 MORSE, P. H., butter slicer, A88
 MORSE, R. E., preserving effect of organic acids, A21
 MOSLEY, W. K., test for quaternary ammonium compounds, A180
 MOSHER, F. T., packaged sundae, A38
 MOSHER, IRA, promoting dealer-consumer understanding, A9
 MUELLER, W. S., sanitizing test cups, A15; stabilization of butter, A132
 MUIR, G. W., urea as protein supplement, A126; digestibility of barley, A173; digestibility of linseed oil meal, A173
 MULDER, H., butter properties, A46; taste compounds of cheese, A47; rennet test, A106; fat globules in buttermilk, A108
 MULLEN, J. E. C., phosphatase test for milk, A166
 MULLER, G., lactose fuchsin-paper disk test for *E. coli*, A32
 MURER, H. K., autoclaving casein, A84
 MURRAY, J. H., chocolate dairy drink, A176
 MUSSETT, A. T., dehydrated sweetened condensed skim milk, A19, A90
 MYRICK, N., brushes for cleaning, A29
- N**ABBEN M., heating milk houses, A139
 NATAF, B., milk cholesterol, A21
 NATH, H., microorganisms in rat cecum, A84
 NELSON, F. E., lactic streptococci bacteriophages, A121
 NELSON, G. L., standards for milk producers, A139
 NELSON, O. M., liquid sugar in food products, A37
 NELSON, W. L., carotene retention in alfalfa meal, A36
 NESBITT, J. M., butter packaging, A105
 NESSENI, R., proteinaceous substances in butter, A60; residual nitrogen of cow's milk, A69
 NEVENS, W. B., soil fertilization and lactation response, A113
 NEWCOMER, E. J., milk vending, A28
 NEWLANDER, J. A., milk flavors, A80
 NICHOLS, AGNES A., inhibitory strains of lactic streptococci, A63; identity of starter streptococci, A64
 NICHOLS, C. W., JR., iodine compounds in fetal bovine thyroid, A148; thyroid gland growth, A148
 NICKELSPORN, H. B., sizing blowoff tanks, A96
 NICKERSON, T. A., packaging print butter, A45
 NILSSON, G., lactic acid from whey, A92
 NITSCHMANN, HS., Ca carbonate particles in milk, A136

- NORD, F. E., ed., *Advances in Enzymology*, vol. IX, A129
- NORDELL, E., water softeners, A36
- NORDEN, J., milk container, A116
- NORTH, A. P., apparatus for breaking and making emulsion, A35
- NORTH, C. E., apparatus for breaking and making emulsions, A35
- O'CONNELL, J., butter-molding apparatus, A1
- O'CONNOR, J. J., flexible couplings, A95
- O'KELLY, RUBY J., phenol-bile media for coliforms, A122
- OLANDER, H. A., cheese-cutting device, A106
- OLANDER, K. E., machine milking plant, A127
- OLDHAM, HELEN, dietary needs of special age groups, A83
- OLSON, H. C., ascorbic acid and oxidized flavor, A22
- OLSON, R. E., HTST pasteurizing system, A51; flow diversion valve, A137
- O'MEARA, J. P., milk filter, A4
- ORLANDO, J., cheese grater, A182
- ORMSBEE, R. W., sulfamethazine for mammary gland infections, A45
- ORRELL, J. B., frozen-cream grinder, A176
- OSER, B. L., food analysis, A71; heat-processing and protein properties, A177
- OTTING, H. E., ion exchange applications, A68; stabilizing evaporated milk, A133
- OTTOSEN, H. E., intradermal brucellosis tests, A44
- OVERMAN, O. R., soil fertilization and lactation response, A113
- OVERMANN, ANDREA, dependability of food judges, A58
- PACKER, R. A., penicillin level in udder, A1; penicillin therapy for mastitis, A1
- PAGONES, J. N., nutritive value of butter, A148
- PALM, W., packaged automatic boilers, A74
- PALMER, F. D., ice cream package-filling mechanism, A183
- PARFENTJER, I. A., casein modification, A136
- PARFITT, E. H., sanitary control methods, A35
- PARKER, M. E., *Food Plant Sanitation*, A43
- PARKER, R. R., Q fever in cattle, A103
- PARKIN, I. E., dairy plant housekeeping, A128; controlling thermophilic bacteria, A164
- PARMELEE, C. E., lactic streptococcus bacteriophage, A121
- PARRISH, D. B., serum vitamin A, A113
- PASSENIER, S. A. H., fat balance sheet, A112
- PATTERSON, V. C., ammonia equipment in ice cream plants, A94; ammonia equipment, A111
- PEARCE, J. A., dried milk products, A120; dried milk, A120
- PEARSON, P. B., niacin metabolism, A142; serum mineral content, A143
- PEAT, R. G., milk salesmen, A141
- PEBBLES, D. D., jet heater, A4
- PELCZAR, M. J., JR., improved medium for milk bacteria, A50; microbacteria, A65; testing quaternary ammonium germicides, A150
- PERKINS, A. G., alarm device for milking machines, A98
- PERKINS, D. G., sulphur preparation for mastitis, A17
- PERLMAR, P. L., hyaluronidase of rat testes, A14
- PERLZWEIG, W. A., niacin metabolism, A142
- PERRY, J. R., cleaning and sterilizing milk tank trucks, A40; cleaning methods, A179
- PERRY, R. L., experimental condensing, A63
- PETERSEN, W. E., milk cholesterol, A21; streptomycin in mammary gland, A119
- PETERSON, M. H., partition chromatography for fatty acids, A182
- PETERSON, W. H., nutrition of *Clostridium sporogenes*, A84
- PETERSON, W. J., phosphate fertilization, A143; milk diets for pigs, A154
- PETTE, J. W., gassy fermentation in processed cheese, A88
- PETCHER, T. J., refrigerated milker pail, A127
- PHILLIPS, G. M., predelivery storage of milk, A38
- PHILLIPS, P. H., colostrum pseudoglobulin, A110; blood serum proteins of calves, A110; tocopherols and milk production, A114; lactation studies, A116; vitamin A and C of ewes and lambs, A149; composition of sow's colostrum and milk, A154
- PHILLIPS, R., seasonal conception rates, A174
- PHILLIPS, T. G., digestible energy of hay, A173
- PICKELL, H. W., ice cream packages, A175
- PIERANGELI, E., brucellosis, A119
- PIERCE, A. E., diagnosis of trichomoniasis, A131

- PIERCE, C. W., milk prices and cost of production, A76
- PLAUT, G. W. E., metabolic regulator in spermatozoa, A148
- PLUM, A., intradermal brucellosis tests, A44
- POE, C. F., germicidal value of glycols, etc., A84; phenolbile media for coliforms, A122; α -methylglucoside fermentation, A122; separation of coliforms, A122
- POLIVKA, D. R., milking barn, A145
- POLIVKA, G. J., milking barn, A145
- POOS, F. W., insecticide studies with dairy cattle, A97
- POPE, A. L., vitamin A and C of ewes and lambs, A149
- POTTER, F. E., whey in sherbets, A186
- POTTER, R. L., colostrum pseudoglobulin, A110
- POTTINGER, F. M., Jr., brucella infections, A151
- POWERS, A. J., filling milk bottles, A176
- PRAEGER, C. W., tote box, A100
- PRELLER, J. H., pasture control, A77
- PRICE, S. G., refrigerating apparatus, A97
- PROUTY, C. C., bacteriophage of cheese starters, A64; lactic bacteriophage inactivation by quaternary ammonium compounds, A166; dairy sanitation with quaternary ammonium compounds, A188
- PUGH, L. H., gonadal hormones and brucellosis, A120
- PRYENSON, H., emulsifying agents in ice cream, A8
- Q**UACKENBUSH, G. G., bottled fresh concentrated milk, A163
- QUAIFE, MARY LOUISE, tocopherols in milk, A108
- QUAM, S. N., butter manufacturing process, A111; butter manufacturing process control, A111
- QUITTER, H. A., sales promotions, for ice cream, A9
- R**ABUFFO, V. M., 1948 ice cream gallonage, A9; ice cream delivery, A112; ice cream in restaurants, A171; self-service ice cream cabinets, A184
- RADEMA, L., rennet test, A106
- RAPOTH, B. A., ice cream carton, A154
- RAGSDALE, A. C., roughage and heifer growth, A142
- RALSTON, N. P., secretion of P³² in milk, A128
- RANGAPPA, K. S., chemistry & mfg. of Indian dairy products, A59
- RAO, C. K., motility of spermatozoa, A6
- RAKER, A. H., polyphosphates and can washing, A117
- REDFERN, R. B., ice cream stabilizers and emulsifiers, A175
- REDIN, E. G., machine milking plant, A27
- REED, J. O., restricted feed intake, A147
- REETZ, C. C. A., marbleized ice cream process, A175
- REGAN, W. M., hereditary digital anomaly, A154
- REID, W. H. E., standardization of dairy processing, A4; consumer preferences in cottage cheese, A132; consumer preferences in cottage cheese, A162
- REINEKE, E. P., process for synthetic thyroprotein, A178
- REINER, M. E., scale stoppage in cooling-water systems, A168
- REISER, R., serum mineral content, A143
- RESUOGAN, J. C. L., detergency, A85
- RICHARDS, T., microbial action on butterfat, A165
- RICE, E. B., milk for lactic cultures, A34
- RIEK, R. F., bovine reactions to hot atmospheres, A84
- RITZMAN, E. G., wood yeast as protein supplement, A24
- RIVERS, W., churn, A4
- ROBBINS, H. H., merchandising ice cream, A112
- ROBERTS, M., heat resistant bacteria in farm water, A34
- ROBERTS, S. J., variations in bovine vaginal smears, A174
- ROBERTS, W. M., dairy plant housekeeping, A29; cultured buttermilk, A107; frozen concentrated milk, A121
- ROBERTSON, A., milk fever, A17, A132
- ROBICHAUX, ROY, selling butter, A46
- ROBINSON, C. H., urea as protein supplement, A126; digestibility of barley, A173; digestibility of linseed oil meal, A173
- ROCKLAND, L. B., chromatography of amino acids, A137
- RODGERS, N. E., enhancing yield of yeast from whey, A93
- ROEDER, G., lactodensimeter for whey, A133
- ROEPKE, M. H., human and bovine brucellosis, A159
- ROESSLER, E. B., triangular taste tests, A58

- ROGERS, E. E., deposition of aerosol particles, A188
- ROGOSA, M., lactose fermentation mechanism, A182
- ROSEN, F., niacin metabolism, A142
- ROSS, R. H., proved sire program, A53
- ROTHSCHILD, LORD, measurement of sperm activity, A97
- ROUNDY, Z. D., cheese making process, A18
- ROWLEY, L., fuels and firing, A123; flue dust problems, A168
- ROWSON, L. E., extracting fertilized bovine egg, A144
- RUDOLPH, W., canned fresh milk, A146
- RUEHE, H. A., dairy foods in diet, A83
- RUSOFF, L. L., nutritional variations in pasture grasses, A6
- SAAL, R. N. J., chemistry and physics of dairy products, A157
- SAMUELSSON, E., churn test, A87; rennet coagulum, A108
- SANDERSON, L., corrosion-resistant metal, A170
- SARKAR, B. C. RAY, amino acids of bovine semen, A114
- SARLES, W. B., microorganisms in rat cecum, A84
- SCARBOROUGH, DOROTHY A., prooxidant effect of ascorbic acid on fat, A167
- SCHAFER, C. H., ice cream packages, A79
- SCHALM, O. W., sulfamethazine for mammary gland infections, A45
- SCHARRES, H. J., economical roof construction, A94
- SCHENCK, L., whey butter, A161
- SCHLINGMAN, A. S., penicillin ointment for mastitis, A160
- SCHMALFUSS, H., determination of fat deterioration, A93
- SCHMIT-JENSEN, H. O., ed., *C. O. Jensen. Selected Papers*, vol. I., A159
- SCHOENLEBER, L. G., ultrasonic energy, A123
- SCHRECK, W. G., carotene concentrates, A77
- SCHROEDER, C. R., sulfamethazine blood concentrations in calves, A57
- SCHUELEK, H. W., production costs for ice cream, A76
- SCHULZ, M., dairy patents, A103; whey beverage, A48; specific gravity of butter, A60; buttermaking procedures, A87; *Manuale Lactis*, A158
- SCHULZ, W., milk receiving, A27; buttermaking procedures, A87
- SCHWARDT, H. H., mange in dairy cattle, A152
- SCHWARZ, G., checking short time heating by phosphatase test, A21
- SCHWARZKOPF, V., milk dump tank, A184
- SCHWEIGERT, B. S., effect of food supplements, A56
- SCOTT, E. C., manufacture of rennet paste, A182
- SCOTT, G. W., calf raising facilities, A54
- SEARING, L. D., direct vs. plate counts, A2
- SEARLES, E. M., insect prevention in dairies. A16; insect and rodent control, A57, A85, A101
- SEATH, D. M., nutritional variations in pasture grasses, A6
- SEEKLES, L., Ca ions and milk instability, A109
- SEELEY, D. B., sanitizing test cups, A15
- SEELY, L. M., cheese cutting machine, A182
- SEGARD, C. P., fat-free milk, A12; milk in human nutrition, A39, A55
- SEKHAR, C. V. C., psychrophilic bacteria, A92
- SHADWICK, G. W., process for making butter, A87; coliforms in ice cream, A92, A164
- SHAW, A. O., breeding failure survey, A53; calf raising facilities, A54; blood ascorbic acid, A57
- SHAW, R. K., quality of churning cream, A161
- SHERILL, M. H., liquid sugar in food products, A37
- SHEURING, J. J., emulsifying agents in ice cream, A8; shrinkage in ice cream, A98
- SHINN, W., power costs, A138; steam costs for heating, A169
- SHIPLEY, J., ice cream costs, A10
- SHIPMAN, R. C., farm milk coolers, A39
- SHIPSTEAD, H., vacuum treatment of milk powder, A22; experimental condensing, A63
- SHOGEN, C. B., mechanical can washing, A73, A122, A167; acid cleaners, A179
- SHULL, G. M., nutrition of *Clostridium sporogenes*, A84
- SIDNEY, R., training milk salesmen, A75
- SILCOX, H. E., review on fermentation, A21
- SINGER, L., retention of radioactive Cu by cattle, A187
- SJÖSTRÖM, G., rennet coagulum, A108
- SKROTZKI, B., fuels and firing, A96, A123
- SMETS, W. Th. G. M., Ca ions and milk instability, A109
- SMITH, A. C., increasing cream viscosity, A27
- SMITH, A. H., secretion of P^{32} in milk, A128
- SMITH, C. R., streptomycin in mammary gland, A119

- SMITH, E. L., immunity transfer by colostrum, A187
- SMITH, F. H., phosphate fertilization, A143
- SMITH, R. H., wetting agents, A28
- SMITH, S. B., frozen confection wrapper, A7
- SMITHOORS, J. F., estrogen treatment, A147
- SNYDER, W. E., emulsifiers in ice cream, A127
- SOMMER, H. H., utilization of solids not fat, A83
- SONNENBERG, M., bovine serum albumen, A110
- SOURKES, T. L., transmethylation of guanidoacetic acid, A149
- SPECK, M. L., cultured buttermilk, A107; *Elementary Experiments in Dairy Bacteriology*, A129
- SPELLMAN, A. F., percheron colostrum, A109
- SPELLMAN, E., scraper for freezers, A122
- SPENCER, G. R., economic importance of mastitis, A45; age and mastitic infection, A104; antibacterial agents for mastitis, A130
- SPENCER, L., factors influencing milk consumption, A22; future of milk consumption, A36; milk dealers profits, A75; milk distribution costs, A125
- SPENCER, R. D., bacteriological examination of milk, A2
- SPIELMAN, A. A., carotene absorption and utilization, A153
- SPIERS, R. J., plastic cream, A19, A48, A107
- SPINK, W. W., epidemiology of brucellosis, A159
- SPRING, H. M., proper pump piping, A169
- SPOULE, W. H., Canadian Cheddar cheese quality, A1; yields of Cheddar cheese, A61
- SPEUNT, D. H., brucella isolation, A121
- STALLCUP, O. T., roughage and heifer growth, A142
- STANKE, F. W., milking machine suspension, A78
- ST. CLAIR, H., apple juice-milk blend, A28
- STEELE, J. H., veterinarians and health, A152
- STEEPLES, G. L., blood glucose level and secretion of adrenal cortical hormones, A188
- STEINER, K., servicing package boilers, A124
- STEVENS, A. H., preparation of dried whey-potato mixtures, A90
- STEVENS, A. R., dairy industry opportunities, A5
- STEVENS, K. R., food poisoning, A86
- STEWART, H. A., milk diets for pigs, A154
- STOENNER, H. G., Q fever in cattle, A103
- STONE, E. C., rumen fermentation, A58
- STOUT, B. E., fat variations in milk, A82
- STREZYNSKI, G. J., centrifugal removal of whey from cheese curd, A72
- STRINGHAM, J. M., whey food, A120
- STROM, JUNIATA, determining iron content of milk, A182
- STRONG, F. M., synthesis of vaccenic acids, A8
- STRONG, K., sales incentives for routemen, A171; improved bill collections, A172
- STUART, L. S., testing quaternary ammonium germicides, A150
- STULTZ, A. W., sustaining penicillin levels, A57
- STUMBO, C. R., spore resistance to high temperatures, A35
- SUTTON, K. H., heat pumps in dairy plants, A138
- SWAIN, P., fuels and firing, A96, A123
- SWENY, L., polysaccharide related to blood group substances, A149
- SWEETMAN, H. L., DDT residue properties, A155
- SWOPE, W. D., viscosity in chocolate ice cream, A99
- AMSMA, A. F., firmness of summer butter, A46; texture of winter butter, A46; differences in firmness of summer butters, A47; factors influencing composition of milk fat, A71; crystallized fat in cream, A93; fat balance sheet, A112
- TABASSUK, N. P., change of milk buffer indices, A67; ice cream emulsifiers, A79
- TARDIFF, R., equipment standards, A101
- TATBER, H., *The Chemistry and Technology of Enzymes*, A43
- TAYLOR, A. W., avirulent brucella strains, A131
- TAYLOR, N. E., centrifugal atomizer, A137
- TERRELL, A. C., paint color in dairy plants, A4
- TENSER, H., determining depth of freezing, A124
- THIESSEN, R., Jr., autoclaving casein, A84
- THOM, E., universal bottles for milk, A10
- THOMA, R. W., nutrition of *Clostridium sporogenes*, A84
- THOMAS, B. F., thermophilic microflora of dairy utensils, A33; thermophilic and thermophilic bacteria in milk "churns," A49
- THOMAS, C. A., strip cup, A115; milker nipples, A115; milking parlor, A154
- THOMAS, R. G., blood ascorbic acid, A57
- THOMAS, S. B., thermophilic microflora of

- dairy utensils, A33; heat resistant bacteria in farm water, A34; psychrophilic bacteria in milk, A34; thermoduric and thermophilic bacteria, A49; incidence of thermoduric organisms, A91; seasonal incidence of thermoduric bacteria, A91; sterilization of milk bottles, A100; thermoduric counts of raw milk, A164
- THOMPSON, R. H. K., artificial protein filaments, A70
- THOMSEN, L. C., dairy operating costs, A51; controlling fat losses, A97; butter workmanship, A104; cost allocation, A141
- THORNE, L. A., lactalbumin preparation, A50
- THORNTON, H. R., quality of churning cream, A161
- TILLERY, H. B., bulk ice cream container, A79
- TING, I., glycerides in butterfat, A108
- TIPPETT, S. G., human brucellosis of bovine origin, A160
- TODOROFF, A., universal agar medium, A91; counting milk bacteria, A134
- TOMPKINS, C., air for dairies, A4
- TOMPKINS, L. T., uniform milk inspection, A81
- TRACY, P. H., homogenized milk problems, A12; keeping quality of dried milk, A62; powdered cream, A63; ice cream stabilizers, A78; washing milk cans, A73; emulsifiers in ice cream, A99
- TREBLER, H. A., detergent evaluation, A40
- TRENTIN, J. J., adrenalectomy of castrated male rat, A13; thiouracil and mammary growth, A128
- TRETSTVEN, W. I., milk composition, A50; proteins, salts, etc. of milk, A50
- TROUT, G. M., rough homogenized milk, A27; Babcock test of reconstituted milk, A71; Babcock test for reconstituted milk, A90; bottled fresh concentrated milk, A163
- TROUT, J. R., fat test for homogenized milk, A166
- TUPENING, A. I., portable milker, A186
- TURNER, C. W., adrenalectomy of castrated male rat, A13; thiouracil and mammary growth, A128; oral effectiveness of thyroxine forms, A148; process for synthetic thyroprotein, A178
- VALENTINE, D., publicity for dairies, A77
- VALETON, F. H., top milk extractor, A11
- VAN DER BURG, P., casein in milk, A108
- VAN DER WAARDEN, M., chemistry and physics of dairy products, A157
- VAN DRIMMELEN, G. C., *Brucella abortus* bovis in milk, A20; brucellosis ring test, A104; east coast fever in S. Africa, A31
- VAN ESSELTINE, WM., cationic germicide activity, A34
- VAN HOON, A., *Sugar: Its Production, Technology and Uses*, A158
- VAUGHAN, L. M., *Farm Work Simplification*, A103
- VERA, H. D., improved medium for milk bacteria, A50
- VETTER, H., piping principles, A95, A123
- VIGILANTE, J., ice cream scoop, A38
- VODAK, L. S., change of milk buffer indices, A67
- VOGEL, E., ed., *Manuale Lactis*, A158
- VOGEL, H. O., centrifugal separator, A35
- VOGEL, M. P., factors influencing composition of milk fat, A70
- VOLKMER, G. H., lactose determination, A166
- VOR, E. A., butyric acid fermentation in cheese, A89
- WAGNER, C. P., heating milk houses, A138
- WALKER, N., psychrophilic bacteria, A92
- WALKER, S. M., estrogen and progesterone treatment, A146
- WALLER, E. F., procaine penicillin G for mastitis, A151
- WARD, A. H., length of gestation, A114; Jerseys in New Zealand, A114
- WARNER, G. S., testing quaternary ammonium germicides, A150
- WARREN, J., triangular taste tests, A58
- WASHKO, F. V., pathogenicity of *Brucella suis* for cattle, A44; collecting sterile blood, A50
- WATSON, C. J., isotope-labelled urea, A126; urea as protein supplement, A126; digestibility of barley, A173; digestibility of linseed oil meal, A173
- WATSON, D. W., dye reduction tests on pasteurized milk, A49
- WATSON, P. D., lactic acid synthetic resins, A22
- WATTS, BETTY M., prooxidant effect of ascorbic acid on fat, A167
- URQUEHART, J. M., binding properties of proteins, A136

- WEBER, A. F., metrorrhagia in virgin heifers, A13
- WEBER, G. A., quaternaries as bactericides, A101
- WEBER, G. R., evaluation of quaternary germicides, A14; sterilization of dishes, A29
- WECKEL, K. G., laboratory control, A23; fat-free vitamin-fortified milk, A81
- WEDEMANN, W., paratyphoid and enteritis bacteria in whey, A49
- WEIDENBURG, J. G., treating casein products, A109
- WEIBY, A. C., milk pail cover, A53; teat cup, A97; milking apparatus, A115
- WEINSTEIN, B., ascorbic acid and oxidized flavor, A22
- WEIR, W. A., chocolate dairy drink, A176
- WEISBERG, S. M., producing devitaminized casein, A152
- WELCH, W. F., advertising problems, A76
- WELDON, W. C., Boston pricing formula, A23
- WELLM, J., fat globule deformation in cream, A48; viscosity of high fat cream, A71; body of butter, A88
- WELSH, M., sulfamethazine blood concentrations in calves, A57
- WENTWORTH, W. A., consumer evaluation of milk, A10
- WEST, G., restaurant sanitation, A40
- WETHERSEE, R. T., percheron colostrum, A109
- WEYBREW, J. A., phosphate fertilization, A143; milk diets for pigs, A154
- WHITE, A. H., aluminum foil for butter, A60
- WHITE, J. C. D., deterioration of dried skim milk, A62
- WHITEHEAD, H. R., moisture retaining characteristics of curd, A60; morphology of *Streptococcus cremoris*, A134
- WHITING, F., placental and mammary tocopherol transfer, A114; tocopherol transfer, A143; tocopherol deficiency of sheep, A154
- WICKERHAM, L. U., carbon assimilation by yeasts, A66
- WILLARD, H. S., storage of cottage cheese, A61
- WILLIAMS, D. H., whey in sherbets, A186
- WILLIAMS, O. B., heat resistance and germicide resistance, A65; selection and heat resistance of spores, A65
- WILLIAMSON, LEROY, refrigeration troubles, A73
- WILLMAN, J. P., tocopherol transfer, A143; tocopherol deficiency of sheep, A154
- WILSON, J. B., *Brucella abortus* 19 identification, A134
- WILSON, M. L., ergothioneine antithyroid activity, A148
- WILSON, R. C., container lid, A26
- WILSMANN, W., theory of centrifugal separation, A138
- WILSTER, G. H., greater cheese sales, A2; manufacturing quality ice cream, A80; vacuator for pasteurization of ice cream mixes, A98
- WINGO, W. J., pancreatin digestion of acetyl proteins, A136
- WINKLER, E. A., Ca and P in cottage cheese, A71
- WINNING, R. J., thermometer well, A153
- WINTERS, L. M., animal breeding, A103
- WINZLER, R. J., vitamin A and thyroid function, A14
- WISE, G. H., serum vitamin A, A113
- WOLF, J., pH of sterilizing solutions, A29; hypochlorite destruction of *S. salivarius*, A49
- WOLFF, J., iodine compounds in fetal bovine thyroid, A148; thyroid gland growth, A148
- WOLLMAN, M. K., bacteriological examination of edible fats, A165
- WOOD, F. W., quality of churning cream, A161
- WYSS, O., heat resistance and germicide resistance, A65
- Y**AGER, N., carotene retention in alfalfa meal, A36
- YEOMANS, A. H., deposition of aerosol particles, A188
- YOUNG, C. I., calf feeder, A37
- YOUNG, R., bonus plan and routemen's sales, A74; annual bonus system for sales, A75; incentives for new customers, A75
- Z**AHM, G. G., evaporation and concentration of liquids, A22
- ZIKA, E., milk strainer, A100
- ZIMMERMAN, M. M., supermarkets, A99
- ZITTLE, C. A., polysaccharide related to blood group substances, A149

SUBJECT INDEX OF ABSTRACTS

ACETATE, as precursor of milk fat, A83
Acetyl methylcarbinol, determination in butter cultures, A2
Acid cleaners, for dairy plants, A179
Acidity, of milk, determination of, A50
Acidity test, for milk, improved indicator for, A68
Acidoproteolytic bacteria, characterization of, A34, A164
Adrenal cortex, role in mammary development, A14
Adrenal cortex secretion, effect of blood glucose level on, A188
Adrenalectomy, effect on mammary gland, A13
Advances in enzymology, vol. IX, book review, A129
Advertising problems, for dairies, A76
Aerosols, of DDT, particle deposition from, A188
Air, for dairy plants, A4
Air filters, for industrial applications, A138
Albumin, bovine serum, interaction of alkyl sulfates with, A110
Alcoholic fermentation, of whey, A21
Alfalfa meal,
 carotene determination in, A23
 carotene retention in, A36; A173
Alloxan, administration to calves, A149
Alpha-methylglucoside, fermentation by coliform bacteria, A122
Aluminum foil, butter packaging with, A60
Amino acids, filter paper chromatography for, A137
Amino nitrogen, of milk proteins, Van Slyke method for, A68
Aminothiazole, goitrogenic effect of, A178
Animal breeding, 4th ed., book review, A103
Animal shelter research, instrumentation for, A144
Annual review of biochemistry, vol. XVIII, book review, A158
Annual review of microbiology, vol. II, book review, A31
Antibacterial agents, *in vitro* action on mastitis organisms, A180
Antithyroid activity, of ergothioneine, A148
Apple juice, concentrated, for milk modification, A28
Arsenic, residual, in tissues of drug-treated cattle, A116

Artificial insemination, sperm activity for, A97
Ascorbic acid,
 for control of oxidized flavor in milk, A22
 in blood, effect of lactation on, A57
 prooxidant effect on aqueous fat systems, A167
Ash determination, conductimetric, for use on lactose, A166
Aureomycin, for treating staphylococcal mastitis, A151
Average plate count ratio, for judging dairy laboratory work, A165
Ayrshires, inheritance of hair curliness, A185

BABCOCK TEST,
 on reconstituted milk, A71, A90
 reader for, A94
Bacteria,
 coliform, in ice cream, A92
 contaminating, in pasteurized milk, A91
 psychrophilic, temperature characteristics of, A92
 thermoduric,
 in farm milk supplies, A91
 modified procedure for determination of, A91
 seasonal incidence in milk, A91
Bacteria counts, on milk, A90
Bacteriophage,
 in cheese starters, A64
 of lactic streptococci,
 action of quaternary ammonium compounds on, A166
 electron micrographs of, A121
Balancing, of business operations, A124
Barley, digestibility of, effect on nutritive plane upon, A173
Benzene hexachloride, effect on milk flavor, A27
Berseem (*Trifolium alexandrinum*), nutritive value of, A153
Bill collections, by milk routemen, A172
Blood,
 of calves, sulfamethazine concentration in, A57
 sterile, method for collection of, A50
Blood group substances, from bovine stomachs, A150
Blowoff tanks, sizing of, A96

- Boilers,
 firing of, A123
 package,
 choice of, A169
 servicing of, A124
 packaged automatic, A74
- Bonus plan,
 for milk routemen, A74, A75
 for sales, A184
- Book reviews,
 Advances in enzymology, vol. IX, A129
 Animal breeding, 4th ed., A103
 Annual review of biochemistry, vol. XVIII, A158
 Annual review of microbiology, vol. II, A31
 Condensed milk and milk powder, 7th ed., A119
 C. O. Jensen, selected papers, vol. I, A159
 Elementary experiments in dairy bacteriology, A129
 Experimental immunochemistry, A159
 Farm work simplification, A103
 Food plant sanitation, A43
 Manuale lactis, A158
 Methods of vitamin assay, A181
 Milchwirtschaftliche Patentberichte, A103
 Milk and milk processing, A43
 Milk industry foundation laboratory manual, 2nd ed., A59
 Monographs on the progress of research in Holland during the war. Chemical and physical investigations on dairy products, A157
 Odors. Physiology and control, A60
 Practical dairy bacteriology, A157
 Pregnancy diagnosis tests: a review, A59
 Principles of food freezing, A43
 Principles of veterinary science, A129
 Some effects of feeding iodinated casein for a long time to cattle, swine and white rats, A130
 Sugar: its production, technology and uses, A158
 The better utilization of milk, A158
 The chemistry and manufacture of Indian dairy products, A59
 The chemistry and technology of enzymes, A43
- Bottles,
 paper, consumer acceptance of, A11
 milk, sterilization of, A100
 universal,
 for milk, A10
 in Columbus, A11
- Bread,
 dried skim milk additions to, A177
 milk solids not fat in, A163
 quality improvement by milk solids not fat, A163
- Breeding failures, causes in Washington, A53
- Brown Swiss, heritable factors for butterfat percentage, A24
- Brucella abortus*,
 effect of gonadal hormones on infection with, A120
 ring test, A44
 stability of avirulent characters of strains 19 and 45/20, A131
 viability in milk samples, A20
- Brucella abortus* (strain 19),
 agglutinin response to vaccination with, A159
 identification by dye bacteriostasis, A134
- Brucella* antibody-complement system, potentiation by sulfonamides, A3
- Brucella* sp., isolation from apparently healthy humans, A121
- Brucella suis*, pathogenicity for cattle, A44
- Brucellosis,
 control in foods, A119
 epidemiologic study in Minnesota, A159
 human and bovine in Minnesota, A159
 immunization against, A131
 nonantigenic allergic agent for intradermal test for, A44
 ring test for milk, A104, A151
 trace minerals for combating of, A151
- Brushes, for cleaning dairy plants, A29
- Building heating, A184
- Bull, expense of, A24
- Bull halter, A54
- Business worth, calculation of, A5
- Butter,
 aluminum foil packaging of, A60
 bacterial discoloration of, A165
 body characteristics of, A88
 butyric acid in, A18
 continuous production of, A35, A72, A111
 controlling body defects of, A60
 cutter for, A161
 diacetyl determination in, A108
 effect of free air in, A105
 improving texture during winter, A46
 increasing firmness during summer, A46
 keeping quality of,
 effect of chemical additions on, A105
 light during manufacture and, A105
 machine molder for, A1

- nutritive value of, at low environmental temperatures, A147
packaging of, A105
packaging of prints, A45
physical aspect of, A31
print cutter for, A32
properties of, influence of fat content of cream on, A46
proteinaceous substances in, A60
selling of, A46
specific gravity and air content of, A60
stabilization against oxidation, A132
structure of, comparison of usual and continuous churning, A181
texture of, control in continuous churning process, A111
variations in summer firmness, A47
water droplets in, A32
water insoluble fatty acids in, A17
workmanship of, A104
Butter, New Zealand, chemical properties of, A70
Butter, summer, growth promoting factor in, A116
Butter, whey, A161
Butter cultures, acetyl-methylcarbinol plus diacetyl in, A2
Butterfat,
 balance sheet for, A112
 correction of butyrometer readings for, A108
 determination of, in milk powder and baby foods, A135
 in buttermilk, A108
Butterfat percentage, heritable factors for, among Brown Swiss bulls, A24
Butterfat production, effect of tocopherols on, A114
Buttermaking,
 alpha continuous process, A161
 comparison of methods, A87
 Fritz process study, A88
 gas saturation process for, A87
Buttermilk,
 butterfat globule size in, A108
 cultured, manufacture from non-fat dry milk solids, A107
Butteroil,
 manufacture of, A163
 technology of, A152
Butyric acid,
 development in cream, A18
 in commercial cream and butter, A18
Butyric acid fermentation, in edam cheese, A89
Byproducts, route sales of, A172
C
CALCIUM, in human diet, from milk, A55
Ca caseinate, electron microscope detmn. of particle size, A136
Calcium salts, effect on renal excretion, A178
Calf feeder, A37
Calf-raising facilities in Washington, A54
Can inverter, for can washer outlet, A137
Can washer, mechanical
 acid and alkaline detergents for, A122
 factors in operation of, A117
 mechanical, operation of, A73
 operation and maintenance, A117
 polyphosphate use in, A117
 study of, A74, A167
Carotene,
 determination in alfalfa meal, A23
 in Norwegian milk and butter, A100
 in pasture grasses, A6
 retention in alfalfa meal, A36, A173
Carotene concentrates, preparation and storage of, A77
Carpet grass, seasonal composition of, A6
Casein,
 devitaminized, solvent treatment process for, A152
 effect of autoclaving with dextrose on nutritive value, A84
 filaments from, treatment of, A109
 in milk, condition of, A109
 in textile fiber, A3, A70
 modification for greater nutritive value, A136
 rennet, treatment for glue use, A48
 solution of, A122
Casein fibers, denaturation in, A167
Casein-formaldehyde dispersion, A69
Cecal flora, rats fed variously, A84
Cheese,
 cutting device for, A106
 grater for, A182
 low temperature storage of, A89
 machine for cutting, A182
 mechanical turning of, A89
 packnging of, A106
 report on sampling, fat and moisture of, A133
 rheological properties of, A106
 sales from new product developments, A2
 statistics of rheological studies on, A106
 taste and flavor compounds of, A47

- Cheese, blue,
punching apparatus for, A48
reasons for *P. roqueforti* dominance in, A66
- Cheese, camembert,
microflora of, A18
ripening of, A18
- Cheese, Cheddar,
bitter flavor in, A61
comparative yields of, A61
cutting for retail trade, A61
envelope for use during curing, A133
extraneous matter in, A106
from pasteurized milk, flavor development of, A61
moisture content and body firmness of, A60
proteolytic enzyme in making of, A18
quality improvement in Canada, A1
rancid flavor due to lipolytic bacteria, A162
rancidity caused by bacteria, A105
- Cheese, cheshire, ripening of, A162
- Cheese, cottage,
Ca and P determinations, on, A71
consumer preferences in, A132, A162
manufacture of low-acid rennet-type, A1
sales contests for, A126
storage of, A61
variations in characteristics, A132
various types of, A120
- Cheese, edam, butyric acid fermentation of, A89
- Cheese, process, gassy fermentation in, A88
- Cheese, roquefort, deficient mold growth in, A162
- Cheese, roquefort-type, lactic starters for use in, A132
- Cheese factories, efficiency in operation of, A74
- Cheese industry,
basic research needed in, A162
future possibilities for, A61
- Cheese manufacture, centrifugal separation of curd in, A72
- Chemical sterilizers, use in dairy industry, A118
- Chlorine sterilizers, present status of, A15
- Chocolate, as coating for ice cream bars, A7
- Chocolate dairy drink, A176
- Cholesterol content, of milk, A21
- Churn,
metallic, A182
small vertical, A4
W45, testing of, A87
- Churning, continuous and usual, butter structure resulting from, A181
- Churns, treatment with quaternaries, A85
- Citric acid, in seminal glands, A173
- Citrus oils, concentrated, analysis of, A79
- Citrus purees, frozen, use in ice cream and sherbets, A187
- Cleaner-sanitizers, advantages and limitations, A179
- Cleaning, of dairy equipment, A117
- Cleaning compounds, control of use, A16
- Cleaning methods, for dairy plants, A179
- Clipping, for cleaner milk, A29
- Clostridium sporogenes*, nutritional requirements of, A84
- Clostridium tyrobutyricum*, in gassy process cheese, A88
- C. O. Jensen, *Selected papers*, vol. I, book review, A159
- Coliform bacteria,
in milk, lactose fuchsin-paper disk method for, A32
in pasteurized milk and milk products, A2
significance in ice cream, A164
- Collections, weekly vs. every-other-week, A52
- Color inheritance, in Shorthorn cattle, A77
- Color selection, of paints for dairy plants, A4
- Colostrum,
feeding of, A6
immunity transfer by, A187
mare's composition of, A109
pseudo-globulin of, A110
- Colostrum feeding, of calves, and blood serum protein electrophoretic studies, A110
- Conception rates, seasonal, A174
- Condensed milk and milk powder*, 7th ed., book review, A119
- Condensing, experimental, problems in, A63
- Confection, frozen, A127
- Consumer solicitation, by routemen, A52
- Cooler,
for cream, A167
for milk in cans, A167
mechanical, for milk, A183
spray, for milk in cans, A39
- Coolers, for milk on farm, A89
- Cooling, of milk, A11
- Cooling water, sulfur dioxide treatment of, A168
- Copper, radioactive, fate after administration to bovine, A187
- Corn starch, influence on roughage digestion, A153
- Corrosion, by Na hypochlorites, A180
- Corrosion resistance of metal, A170
- Corynebacterium pyogenes*, in bull semen, A78

- Cost allocation, in multiple product plants, A141
- Cost control, for dairy plants, A184
- Couplings, flexible, A95
- Cream,
 apparatus for separation and homogenization of, A35
 butyric acid in, A18
 defects of, causes and preventive measures, A27
 development of butyric acid in, A18
 fat content of, influence on butter properties, A46
 for buttermaking, cooling of, A60
 for churning, quality of, A161
 4-day grading program for, A105
 improvement program for, A105
 production and delivery of, A82
 reseparation method for increasing viscosity of, A27
 sediment test standards for, A150
 water insoluble fatty acids in, A17
- Cream, 80%, deformation of fat globules in, A48
- Cream, frozen,
 block grinder for, A176
 storage of, A25
- Cream, high-fat, viscosity measurements on, A71
- Cream, homogenized, bicarbonate addition to, A100
- Cream, pasteurized, bacterial flora and keeping quality of, A82
- Cream, plastic,
 manufacture of, A163
 production and uses, A19
 storage of, A25
- Cream, powdered, manufacture of, A63
- Cream, whipped, ready-to-use from cans, A146
- Cream dispenser, A54
- Cream remover, for milk bottles, A11
- Creameries, suggested improvements of, A73
- Crossbred dairy calves, growth, A174
- Cysteine, prooxidant effect of aqueous fat systems, A167
- DAIRY EQUIPMENT,**
 cleaning of, A117
 sanitary standardization of, A101
- Dairy farm, sanitation for, A15
- Dairy heifers, growth on roughages, A142
- Dairy industry, opportunities in, A5
- Dairy plants,
 acid cleaners for, A179
 cleaning methods for, A179
 cost control for, A184
 efficient operation of, A74
 housekeeping for, A128
 multiple-product, A94
 operating costs in, A51
 planning, construction and maintenance of, A72
 production costs in, A172
 wastes and losses in, A172
- Dairy research,
 in agricultural experiment stations, A57
 under the Research and Marketing Act, A58
- Dairy sanitation, progress in, A16
- Dallis grass, seasonal composition of, A6
- DDT,
 as spray residues on foods, determination of, A150
 determination in milk, A3
 failure to control houseflies, A155
 in aerosols, deposition of particles from, A188
 in milk of sprayed cows, A186
 residual properties of, A155
 use on cattle, A97
- Dealer education, for ice cream sales, A52
- Dehorning,
 painless, A78
 solution for, A6
- Depreciation reserves,
 adequacy of, A124
 maintenance of, A53
- Detergents,
 characteristics of, A85
 for dairy plants, use and evaluation of, A40
 synthetic, new developments in, A128
 synthetic non-ionic, in combination cleaner-sanitizers, A40
- Diabetes, pancreatic, in calves, A149
- Diacetyl,
 determination in butter, A108
 determination in butter cultures, A2
- Digital abnormality, hereditary, in cattle, A154
- Diplococcin, characterization of, A20
- Direct microscopic count,
 comparison with plate counts, A2
 on pasteurized milk, A90
 preparation of smears for, A20
 staining of smears for, A163
- Diarrhea, sterilization of, A29
- Dishwashing, high-temperature, A101
- Dried milk products, keeping quality of, A62

Drier,
centrifugal spray, for milk, etc., A136
for milk, etc., A137
spray, for milk, A183
Dry ice, for ice cream refrigeration, A26
Drying, spray, problems in, A63
Dump can, for milk, A184
Dust, types of material in, A30
Dwarf cattle, for the tropics, A154
Dynamometer, for determining depth of freezing in foods, A124

EAST COAST FEVER, in South African cattle, A31

Egg composition, containing milk, A163
Eggs, frozen, pathogens in, A92
Electricity, cutting farm costs with, A7
Electron bombardment, for milk sterilization, A51
Elementary experiments in dairy bacteriology, book review, A129
Emulsifiers,
effect on physico-chemical properties of ice cream, A8
for ice cream, A8, A79, A98, A127, A175
Emulsions, device for making and breaking of, A35
Enteritis bacteria, in whey, A49
Entrainment separator, for vacuum pans, A4
Equipment, dairy, sanitary design of, A35
Ergothioneine, antithyroid activity of, A148
Escherichia and *Aerobacter*,
growth on phenol-bile media, A122
separation with boric acid medium, A122
Estrogens,
effect on rat lactation, A146
effect on rat mammary structure, A147
Experimental immunochemistry, book review, A159
Extraneous matter, in Cheddar cheese, A106

FARM DAIRIES, grade A, design standards for, A139

Farm work simplification, book review, A103
Fat,
crystallized, determination in cream, A92
globular and free, in Fritz process butter, A88
Fat deterioration, determination of, A93
Fat globules, in butter, measurement of, A104
Fat losses, control of, A97
Fat test, Babcock, for homogenized milk, A166

Fat variations, in milk, A82
Fats, edible, bacterial examination method for, A185
Fatty acids,
estimation by chromatography, A182
production in butter by microorganisms, A165
water-insoluble, in cream and butter, A17
Feedwater, treatment of, A124
Fermentation processes review, A21
Filter, for milk, A4
Fieldmen, role in producing sanitary milk, A117
Flavor materials, for ice cream, A7
Flow diversion valve, for control of HTST pasteurization, A137
Flue dust, control of, A168
Folic acid activity, of milk, microbiological determination of, A136
Food analysis, review of methods for, A72
Food plant sanitation, book review, A43
Food poisoning, A86
Food technology, symposium on, A41
Forage crops, management for higher yields, A24
Frost little plate method, modified, for milk, A134
Fuels and firing, of boilers, A96

GASSY FERMENTATION, in process cheese, A88

Genes, major, in evolution of economic characters, A78
Germicides, cationic, activity of, A34
Gestation, length of, in N. Z. dairy cattle, A114
Globular fat, in butter, dilution method for measurement of, A104
Globulin, gamma electrophoretic-convection fractionation of, A179
Globulins, immune, in plasma of hyperimmunized cows, A110
Glucose, blood level of, effect on adrenal cortex secretion, A188
Glycerides, fully-saturated, determination in N. Z. butter, A108
Glycol benzoates, germicidal activity of, A34
Glycols, germicidal activity of, A34
Goitrogenic effect of aminothiazole, A178
Goodwill, estimation of, A52
Guanidoacetic acid, transmethylation in beef liver autolysates, A149

HALTER, breaking lead type for cattle, A54
Hays, composition and digestible energy of, A173
Heat,
 effect on Jersey calves, A84
 effect upon Jerseys in milk, A84
Heater, steam, for liquids, A4
Heating,
 of buildings, A184
 relation to steam costs, A169
Heating pumps, for the dairy industry, A188
Heating rates, of foods in containers, A36
Homogenization, of a portion of an ice cream mix, A25
Homogenizer,
 centrifugal, A184
 new, A72
Horn flies, insecticides for control of, A185
House flies,
 failure of DDT to control, A155
 toxicity of synthetic pyrethrins to, A155
House heating, by cows, A186
Housekeeping, in dairy plants, A29
Hyaluronidase,
 in bull spermatozoa, A6
 in rat testes, A14
Hypocalcemia, bovine, Sulkowitch test for, A178
Hypochlorite aerosols, critical humidity for bactericidal action of, A49
Hypochlorite solutions, efficacy at different pH levels, A29
Hypochlorites, sodium, corrosion by, A180
Hypoderma sp., occurrence in cattle, A161

ICE CREAM,
 bulk container for, A79
 coliform bacteria in, A164
 combination offer of, A9
 consumer survey on merchandising of, A52
 container for, combined spoon and lid for, A27
 control of finished product, A9
 cost system in manufacture of, A170
 costs in manufacture and sale of, A10
 creation of marbled patterns in, A175
 dealer education for sales of, A52
 dealer pricing for profits, A52
 dealer service costs for, A184
 defects in, A115
 defects of, curing of, A175
 delivery to stores, A112

 drug-store merchandising of, A112
 emulsifiers for, A8, A79, A98, A127
 flavor and texture preferences, A174
 flavor preference tests for, A26
 flavoring materials for, A7
 gallone analysis, A187
 gallone for 1st half of 1948, A9
 gallone for 1948, A54, A80
 gallone for May, 1949, A146
 home market for, A140
 improved cone for, A115
 in restaurants, A171
 in supermarkets, A99
 in theaters, A140
 increasing sales of, A112, A170
 liquid sugar use in, A7
 manufacture of, quality requirements of, A80
 nutritive value of, A83
 package filler for, A183
 package trends for, A79
 packaged sundae, A38
 packaging of, A115, A175
 physico-chemical properties of, effect of emulsifiers on, A8
 pint bowls for packaging of, A99
 production costs, of, A76
 promoting dealer-consumer understanding, A9
 quality in manufacture of, A80
 refrigeration requirements for manufacture of, A51
 sales of, for January, 1949, A99
 sanitation in production and handling of, A85
 scoop for, A38
 self-service and package merchandising of, A112
 self-service stores for, A140
 selling cost calculator, A26
 selling through vending machines, A37
 shrinkage of, A98, A186
 stabilizers and emulsifiers for, A175
 stabilizers for, A79
 stimulating sales of, A9
 texture of, control with microscope, A187
 use of laboratory for control of, A9
 wholesale prices of, A113
 wind tunnel for hardening of, A25
Ice cream, chocolate coated, A7
Ice cream, diced, A54
Ice cream, fried, A25
Ice cream cabinets,
 relation to sales and profits, A80

self-service, in drug stores, A184
 standard dimension of, A145
 Ice cream eclairs, A79
 Ice cream freezer,
 agitator for, A187
 scraper for, A122
 Ice cream gallonage, shifts in, A145
 Ice cream industry, unethical practices in,
 A140
 Ice cream mix,
 cooling of, A37
 homogenization of a portion of, A25
 HTST pasteurization of, A98
 superheating of, A25
 vacreator pasteurization of, A98
 Ice cream mix, chocolate, viscosity of, A99
 Ice cream mix, concentrated, storage of, A25
 Ice cream mix, frozen, storage of, A25
 Ice cream package, A154
 Ice cream packer, A54
 Ice cream plant, work simplification in, A125
 Ice cream "snowballs," A140
 Ice cream specialty, cake roll, A145
 Ice cream trucks, refrigeration for, A168
 Ices, making of, A175
 Immunity, transfer to new-born calf in colostrum, A187
 Incentive plan, for milk sales, A53
 Infant food, powdered, from cows' milk, A121
 Infertility, of Friesian bulls, inherited seminal character in, A144
 Insecticides,
 food adulteration with, A155
 for horn fly control, A185
 organic,
 fumigating properties of, A155
 residual activity of, A155
 Insects,
 control in dairy plants, A16, A57, A85
 Inspection, uniform, for milk supplies, A81
 Insulation, of cold rooms, A94
 Investment return, adequacy of, A75
 Iodinated casein, long-time feeding of, A130
 Iodine number, of New Zealand butter, A70
 Ion exchange, application to milk products,
 A68
 Iron, in milk, determination of, A182

J

JERSEYS,
 effect of heat on, A84
 N. Z. breed type and production records of,
 A114

Judging, of food,
 dependability of, A56
 triangular taste tests for, A58

K
KARAKUL-type hair curliness, of Ayrahire
 cattle, inheritance of, A185
 Kefir, biological value of proteins in, A56
 Kjeldahl method, for milk proteins, A68

LABOR RESPONSIBILITY, in future development of dairy industry, A74
 Labor saving, on farm, with electricity, A7
 Laboratory, plant, for ice cream control, A9
 Laboratory control, of finished dairy products,
 A23
 Lactalbumin, treatment for resuspension of,
 A50
 Lactation,
 effect of steroids on, A13
 effect on blood ascorbic acid, A57
 regulation of, A28
 Lactation, of rats, on natural and purified
 diets, A116
 Lactation response, influence of soil fertilization on, A113
 Lactic acid,
 from whey, A92
 polymers of, as synthetic resins and coatings, A22
 Lactic starters, for Cheddar cheese, influence
 of penicillin on, A134
 Lactic streptococci, influence of abnormal milk
 on activity of, A34
 Lactodensimeter readings, on whey, correction
 for, A133
 Lactose,
 ash determination in, A166
 detmn. in milk products, A166
 fermentation by yeasts, mechanism of, A182
 methanol extraction of, from skim milk
 powder, A135
 Lactose, crude, preparation of, A163
 Lactose, low-ash, manufacture of, A107
 Lecithin, crude soybean, effect on vitamin A
 absorption, A153
 Leptospira, bovine, comparison of N. J. and
 Palestine strains, A119
 Lifter, for milk cans, A167
 Linseed oil meal, digestibility of, A173

M
MALTED MILKS, sales and profits of,
 A145

- Mammary development, role of adrenal cortex in, A14**
- Mammary gland,**
adrenalectomy and the, A13
growth of, thiouracil effect on, A128
infusion with streptomycin, A119
sustained penicillin level in, A57
- Mammary gland infections, sulfamethazine for treatment of, A45**
- Mange, in dairy cattle, control and treatment of, A152**
- Manuale lactis*, book review, A158**
- Manure, reinforcement with superphosphate, A78**
- Mastitis,**
age and infection in, A104
detection, control and prevention of, A44
early features of, A17
hemolytic group C streptococci in, A160
optimal testing of milk for, A87
organisms of, antibactericidal action on, A130
penicillin therapy for, A1
procaine penicillin G for treatment of, A151
review on treatment of, A152
staphylococci, aureomycin treatment of, A151
sulfamethazine and penicillin for treatment of, A151
sulfa preparations for, A17
treatment with penicillin ointment, A160
- Mastitis, in England, infective agents for, A45**
- Media, for microbial counts on milk, A134**
- Medium, universal, for bacteria, A91**
- Methods of vitamin assay*, book review, A181**
- Methylene blue test,**
for dried milk, A66
for pasteurized milk, A49
- Metrorrhagia, in virgin heifers, A13**
- Microbacteria, characteristics of, A65**
- Micrococci, of milk, classification of, A65**
- Microorganisms, taxonomy of, A134**
- Milchwirtschaftliche Patentberichte*, book review, A103**
- Milk,**
acidity of, A66
acidity of, improved indicator for, A68
acidity of, standardization of methods of determination of, A50
antioxidant gas treatment of, A93
as human food, A55
bottle-filling measurements for, A176
Brucella abortus ring test for, A44
buffer indices of, A66
cholesterol content of, A21
coliform bacteria in, A32
composite sampling of, A3
composition of,
changes in, A67
ratio of fat to other constituents, A67
relationships between constituents, A67
consumer evaluation of, A10
consumption of, in future, A22
cooling of, A11
DIT in, A3
delivery of, controlling costs of, A125
distribution of,
costs and profits in N. Y., A125
possible economies in, A171
farm coolers for, A39
fat-free vitamin-fortified, A81
flavor of, effect of benzene hexachloride on, A27
flavors in, A80
fortification with vitamins A and D, A28
grading of,
resazurin test for, A2
resazurin test plus direct microscopic count for, A2
home delivery of, A141
home pasteurization of, A81
hygiene of, A20
inhuman diet, A55
increasing human consumption, A55
instability of, Ca ions in, A109
iron determination in, A182
keeping quality of, visual conditions on farm and, A38
mechanical cooler for, A183
media for microbial counts on, A134
modification with concentrated apple juice, A28
modified Frost little plate for use on, A134
nutritive value of, A39; A116
nutritive value of, animal evaluation for, A56
optical testing for mastitis in, A87
oxidized flavor in, control with ascorbic acid, A22
pasteurization and safety of, A135
pasteurization of, A55
pricing of, Boston formula for, A23
proteins in, Kjeldahl method modifications for, A68
psychrophilic bacteria in, A34
quality tests for, A175
receiving of, A27
resazurin test for, A32

- Milk—continued**
sediment test standards for, A150
spray cooling in cans, A39
spray drier for, A183
strainer for, A100
supplementation of, for pig diets, A154
tank truck hauling of, from farm to plant, A146
technical advances in handling of, A12
thermoduric bacteria in, A33
3-day delivery of, A99
uniform inspection of, A81
universal bottles for, A10
vacuum concentration of, A22
variations in fat and total solids, A50
variations in proteins, salts, etc., A51
vending of, A28
- Milk, abnormal, and acid production by lactic streptococci, A34**
- Milk, canned, new process for, A146**
- Milk, chocolate,**
making of, A12
milk fat in, A27
- Milk, concentrated,**
fortification of, A182, A183
for use in ice cream, A63
- Milk, concentrated fresh, consumer reaction to, A163**
- Milk, concentrated frozen, A121**
- Milk, dried,**
cost of manufacture, A113
dye-reduction tests for, A66
factors in manufacture of, A120
- Milk, dried skim, addition to bread, A177**
- Milk, evaporated,**
canning method for, A163
stabilization of, A133
turning machine for, A49
- Milk, fat-free,**
as special dairy product, A12
selling of, A38
- Milk, fat variations in,**
dairy plant factors, A82
farm factors, A82
- Milk, frozen condensed, storage of, A25**
- Milk, homogenized,**
fat test for, A166
problems of, A12
rough or spotty defect of, A27
- Milk, pasteurized,**
direct microscopic count for, A90
dye reduction tests for, A49
keeping quality of, A38
significance of coliform bacteria in, A2
- Milk, powdered,**
moisture removal from, A90
use in cultured buttermilk, A107
- Milk, quick-frozen homogenized, A128**
- Milk, reconstituted, Babcock fat test for, A71, A90**
- Milk, spray-dried, bacterial growth after reconstitution of, A66**
- Milk, sweetened condensed, bacteriology of, A49**
- Milk and milk processing, book review, A43**
- Milk bottle,**
cream separator for, A176
paper, A55; A116
paper, cap for, A27
- Milk can inverter, A116**
- Milk cans,**
comparison with tank trucks, A11
lifting hoist for, A153
seasonal variations in sterility of, A101
thermoduric bacteria in, A33
thermoduric and thermophilic bacteria in, A49
- Milk code, U.S.P.H.S., recent amendments of, A38**
- Milk consumption, future of, A36**
- Milk dealers, profits of, A75**
- Milk delivery tube, for use in cans, A27**
- Milk fat,**
acetate as precursor for, A83
factors influencing composition of, A70
manufacture, packaging and use, A18
- Milk fever,**
blood minerals in, A132
observations on, A17
- Milkhouse, heating of, A139**
- Milk ordinances, effectiveness of, A10**
- Milk pail, refrigerated, A127**
- Milk pail cover, A53**
- Milk plants,**
"break-even" point in operation of, A141
business charts for management of, A171
cooperatively built, A5
cost reduction in, A171
efficient planning of, A73
helpful accessories for, A127
layout of, A72
municipally owned, A10
problems in operation, A52
- Milk powder,**
skim, changes in storage, A62
spray-dried, bacteriological quality of, A62
vacuum treatment of, A22
whole,

- for ice cream and milk plants, A111
- for ice cream plants, A94, A123
- Refrigeration, freon, ice cream plant, A86
- Refrigeration requirements, for ice cream manufacture, A51
- Refrigerators, insulation of, A95
- Raichebt values, of New Zealand butter, A70
- Renal excretion, effect of Ca salts on, A178
- Bennet coagulum, elastic properties of, A108
- Bennet paste, manufacture of, A182
- Bennet test, direct method of Van Dam, A106
- Resazurin test,
 - for dried milk, A66
 - for milk, A32
 - for pasteurized milk, A49
 - milk grading with, A2
- Restaurant sanitation, A41
- Restraining device, for cattle, A54
- Retail milk delivery, 3-day, A75
- Rodents,
 - control in creameries, A85
 - control in dairy plants, A57
- Roof construction, choice of, A94
- Roughage digestion,
 - influence of corn starch on, A153
 - influence of protein level on, A142
 - influence of soybean oil meal on, A142
- Roughages, for growing dairy heifers, A142
- Routemen,
 - bonus plan for, A74, A75
 - milk,
 - bill collections by, A172
 - bonuses for increased sales by, A171
 - collection incentives for, A113
 - homogenized milk sales by, A171
 - sales contest for, A171
 - wages paid, A141
- Rumen fermentation, under normal and atonic conditions, A53
- SAFETY**, in industrial plants, A139
- Sales contest, for milk plants, A126
- Salesmen,
 - milk, training of, A75
 - training of, A141
- Samples, composite, semi-monthly vs. monthly, A3
- Sanitary design, of dairy equipment, A35
- Sanitary standardization, of dairy equipment, A101
- Sanitation, for ice cream, A85
- Saponification values, of New Zealand butter, A70
- Scoop, for ice cream, A38
- Sediment, in cream, dust and fine debris in relation to, A30
- Sediment test standards, for milk and cream, A150
- Sediment tester, vacuum, for milk, A100
- Selenium,
 - distribution in milk proteins, A188
 - passage through rat mammary gland, A188
- Selling, prying for profit, A74
- Semen, bull,
 - amino acid composition of, A114
 - Corynebacterium pyogenes* in, A78
 - hyaluronidase activity of, A6
 - metabolic activity of, A153
 - methods of evaluation of, A53
- Seminal character, inherited by infertile Friesian bulls, A144
- Seminal glands, fructose and glucose in, A173
- Separator, centrifugal,
 - for cheese curd separation, A72
 - self-washing, A35
 - with raisable supply tank, A183
- Separator bowl, theoretical discussion of action, A138
- Separator-homogenizer, for cream, A35
- Serum proteins, bovine, electrophoretic fractionation of, A150
- Shaft alignment, checking of, A170
- Sherbets,
 - making of, A175
 - why use in, A186
- Shorthorns, color inheritance in, A77
- Shrinkage, of ice cream, A98, A186
- Silage, from steamed potatoes, bacteriological changes in, A66
- Skim milk,
 - acidification for feeding, A185
 - dried, egg substitute from, A109
- Skim milk powder, lactose and soluble protein extraction from, A135
- Slow accounts, collection of, A184
- Soil fertilization, influence on lactation response, A113
- Sow's colostrum and milk, composition of, A154
- Soybean oil, influence on bovine roughage digestion, A142
- Sperm, activity of, measurement, A97
- Spermatozoa,
 - effect of dilution on motility and fertility, A144
 - metabolic regulator for, A143
 - motility of, A6

- Spores, bacterial,
 increasing heat resistance by selection, A65
 resistance to heat and germicides, A65
 technic for temperature resistance of, A35
Spray drier, for milk, A122
Stabilizers, for ice cream, A79, A175
Stains, for direct microscopic count of milk,
 A163
Staphylococci,
 sensitivity to penicillin, A160
 of milk, classification of, A65
Starters,
 penicillin action on, A107
 selection of lactic streptococci for, A63
Starters, cheese, bacteriophage in, A64
Starters, lactic, for use in roquefort-type
 cheese, A132
Steam costs, for dairy plants, A138
Sterilizers, chemical, present status of, A15
Steroids, effect on lactation, A13
Strainer, for milk, A100
Streptococci,
 hemolytic group C, in bovine mastitis, A160
 lactic,
 for starters, identity of, A64
 inhibitory strains of, A63
 of milk, classification of, A64
Streptococcus agalactiae, infections by, peni-
 cillin for, A130
Streptococcus cremoris,
 degeneration in starters, A92
 inhibitory strains of, A63
 inhibitory substance produced by, A20
 morphological differences in, A134
 penicillin inhibition of, A164
Streptococcus lactis,
 degeneration in starters, A92
 inhibitory strains of, A63
 inhibitory substance production by, A20
 penicillin inhibition of, A164
Streptococcus salivarius, critical humidity for
 destruction by hypochlorite aerosols, A49
Streptomycin, effect of infusing mammary
 gland with, A119
Strip cup, A115
Strychnine, residual, in tissues of drug-treated
 cattle, A116
Sugar: Its Production, Technology and Uses,
 book review, A158
Sugar, liquid,
 in food products, A37
 use in ice cream, A7
Sulfamethazine,
 for treatment of mammary gland infections,
 A45
 in blood of calves, A57
Sulfamethazine plus penicillin, for mastitis
 treatment, A151
Sulfanilamide, for mastitis, A17
Sulfur dioxide, for treatment of cooling
 water, A168
Sulkowitch test, for bovine hypocalcemia,
 A178
Superphosphate, for reinforcing farm manure,
 A78
Sweetened condensed skim milk, dehydrated,
 manufacture, storage and use of, A19

I ANK TRUCKS,
 for hauling milk from farm to plant, A146
 for milk, A11
Taxes, keeping at a minimum, A23
Teat, draining tube for, A6
Teat cup, for milking machines, A97, A145
Testosterone propionate, effect on spermat-
 ogenesis, A174
The better utilization of milk, book review,
 A158
The chemistry and technology of enzymes,
 book review, A43
Thermoduric bacteria,
 control of, A164
 in cheese making, A65
 in farm water supplies, A34
 in milk, A33
 in milk cans, A33, A49
 in raw milk, A164
 of dairy utensils, A33
Thermometer, mounting well for, A153
Thermophilic bacteria, in milk cans, A49
Thiouracil, and mammary gland growth, A128
Thyroid function, influence of vitamin A de-
 ficiency on, A14
Thyroid gland, of bovine fetus,
 growth of, A148
 iodine compounds in, A148
Thyroprotein, synthetic production of, A178
Thyroxine, oral effectiveness of, A146, A148
Tocopherol, deficiency of, among sheep, A154
Tocopherols,
 determination in human milk, A108
 effect on mammary and placental transfer
 of vitamin A, A143
 effect on milk and butterfat production,
 A114

. placental and mammary transfer of, A114
Tete box, for milk handling, A100
Trap discharge lines, A139
Treponema theileri, transmission from donkey to calf, A31
Trichomonas foetus, treatment of bulls infected with, A44
Trichomoniasis,
 bovine, mucous agglutination test for, A131
 bovine venereal, field studies on, A44
Trucks, for ice cream, moisture accumulation in, A26
Truck bodies, undercoating of, A86
Tuberculosis, human, bovine origin of, A160

ULTRASONIC ENERGY, use in agriculture, A123

Undercoating, of truck underbodies, A86
Universal milk bottle plan, in Columbus, A11
Urea,
 as protein supplement, A126, A141
 isotopically labelled,
 body nitrogen formation from, A126
 synthesis of, A126
U.S.P.H.S. milk code, amendments of, A38
Utensils, dairy, thermophilic bacteria in, A33

VACCENIC ACID, synthesis of, A3
Vacreation, for pasteurization of ice cream mix, A98
Vacuum pans, entrainment separator for, A4
Vaginal smear, of cow, variations in, A174
Van Slyke method, for free amino groups in milk, A68
Vending machines, for ice cream, A37
Veterinarians, relation to community health, A152
Vibrio foetus, infection of cattle with, A31
Viscosity, of ice cream mix, A99
Vitamin A,
 absorption of, effect of crude soybean lecithin on, A153
 effect on thyroid function, A11
 effects of feeding on blood serum values, A113
 in Norwegian milk and butter, A100
 milk fortification with, A28
 of ewes and lambs, A149

 mammary and placental, transfer of, influence of tocopherols on, A143
 Vitamin C, of ewes and lambs, A149
 Vitamin D,
 assay in milk, A83
 milk fortification with, A28

WASTES AND LOSSES, in dairy plants, A172

Water,
 added, detection by serum tests, A135
 on the farm, thermophilic and thermophilic bacteria in, A34
Water cooling tower, upkeep of, A167
Water softeners, use and care of, A36
Water supply, from tank, for milk cooling, A167
Watering device, for livestock, A174
Watering fountain, for stock, A154
Wetting agents, use in dairy plants, A28
Whey,
 alcoholic fermentation of, A21
 determination of quality, A48
 fermented beverage from, A48
 filtration for human use, A133
 history of milk sugar from, A48
 in sherbets, A186
 lactic acid from, A92
 lactodensimeter corrections for, A133
 paratyphoid and enteritis bacteria in, A49
 proteins of, from various animals, A109
 yeast yield from, A93
 dried, browning and staling prevention in, A121
Whey food products, making of, A120
Whey-potato mixtures, dried, preparation of, A90
Whey protein, denaturation by heat, A69
Wind tunnel, for hardening ice cream, A25
Work simplification, in ice cream plants, A125
Wrapper, for frozen confections, A7

YEAST,
 from whey, A93
 lactose fermentation mechanism for, A182
 wood, as protein supplement for cattle, A24
Yeasts, carbon assimilation tests in classification of, A66

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or before

Return Date	Return Date